Chapter 10. Introduction to the symmetrical network theory

In chapter 8 I described the Richter theory, the first mechanistic theory to be developed based on Jerne's immune network hypothesis. In chapter 9 I made the detailed case that V-V interactions are symmetrical rather than asymmetrical. I now describe a second mechanistic immune network theory, that incorporates symmetric interactions between idiotypes and antiidiotypes. The first paper was published in 1975, at a time when the dominant point of view favoured asymmetric rather than symmetric V-V interactions.[111] After the basic aspects of the model were formulated, a search of the literature revealed that a lot of published data supported the symmetric theory. It was a very exciting experience, as more and more published data was found that fitted the emerging theory, and permitted it to be refined.

Idiotypes, antiidiotypes and symmetry

As a first approximation, cells of only two specificity classes were initially included in the basic version of the symmetrical theory. This aspect was emphasized by using a "plus-minus" nomenclature as follows. Lymphocytes that have receptors specific for the antigen (antigen-specific idiotypes) are called positive cells, while cells with receptors specific for the receptors on positive cells (antiidiotypic cells) are called negative cells. More broadly, positive cells can be interpreted as all those cells whose receptors are more complementary to the antigen than are similar to (resemble) the antigen, while negative cells are the ones whose receptors resemble the antigen more than they are complementary to the antigen. The broader definition suggests that all the cells of the repertoire are either positive or negative with respect to a given antigen. Thus two large fractions of the repertoire may be directly or indirectly involved in the regulation of each immune response, just different particular fractions for each antigen.

B cells and T cells of the two specificities are written B_+, B_-, T_+, and T_- respectively. In this model the terminology emphasizes the symmetry in the interactions and the fact that cells stimulated by "Ab-2" cells are expected to be mainly "Ab-1" cells rather than "Ab-3" cells (as defined by Richter), because Ab-1 is stimulated by both Ab-2 and the antigen, while Ab-3 is stimulated only by Ab-2.

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[111] G. W. Hoffmann (1975) “A network theory of the immune system.” Eur. J. Immunol., 5, 638-647, 1975.

Three kinds of specific, symmetrical interactions

The symmetrical network theory is more strongly based on explicit mechanisms than it's two predecessors. Like the Richter theory, it includes stimulatory, inhibitory and killing interactions, but the interactions are based on more defined molecular-cellular mechanisms.

The cross-linking of receptors is postulated to be an activation mechanism for both B cells and T cells, and we have seen how the symmetry of stimulatory interactions follows from this postulate. Extensive data support the cross-linking of receptors and symmetry in stimulation, especially for B cells, as discussed in chapter 9. Lymphocytes themselves are efficient antigens, since they have a large number of specific receptors (about 10^5) on their surfaces that can cross-link receptors on another lymphocyte. Two lymphocytes with mutually complementary receptors ("positive" and "negative" cells, or populations “1” and “2” respectively) can be expected to stimulate each other to proliferate or to secrete their particular specific molecules (antibodies or specific T cell factors).

The antigen-specific T cell factors described in chapter 6 play an important role in the symmetrical network theory. There is an extensive literature on such factors, published mainly in the 1970s and early 1980s. Since they have a molecular weight of approximately 50,000, it is postulated in the theory that they are monovalent with respect to antigen binding. (Antibodies with a molecular weight of 150,000 have two antigen binding sites.) A monovalent factor in soluble form could not cross-link receptors that are complementary to itself, whereas it could block such receptors. In the absence of any information to the contrary, we make the reasonable and simplifying assumption that this blocking is symmetric; positive factors block negative receptors and vice versa. A model with asymmetric blocking would be more complex, and therefore inferior from the point of view of maximizing simplicity (see 1).

Antibodies of a given specificity eliminate (kill) cells with the complementary specificity. In chapter 2 the two main classes of antibody, called IgM and IgG, were described, that kill cells by using a set of serum proteins called complement. There is also antibody dependent cellular cytotoxicity (ADCC) in which the Fc region of antibodies (with, say, positive specificity) bind to Fc receptors on such as macrophages, and the macrophages are then able to engulf cells with the negative specificity. A third form of killing is by the class of T cells called cytotoxic T cells. In all cases we can most simply, and thus most reasonably, assume that if positive antibodies or receptors are effective in eliminating negative cells, the converse should also be true.

An aside on stability

Stability analysis is a powerful tool for the analysis of dynamical systems. A steady state of a system of differential equations may be stable, unstable or neutrally stable in each of the relevant variables, as is illustrated for some simple systems in Figure 10-1. A block sitting on a flat smooth surface is stable in both of two dimensions, meaning it returns to a flat position if tipped by a small amount in either the x or the y direction. The block is then at an "attractor". A cone balanced on its point is unstable in two dimensions and it is then at a "repellor". A dead man sitting in a saddle may be stable with respect to falling off the horse forwards or backwards, but is unstable with respect to falling to one side or the other. A steady state of a dynamical system that is stable in one direction and unstable in a second direction is accordingly called a "saddle point". A sphere on a flat smooth surface is neutrally stable in two dimensions, meaning if a small perturbation moves it a small distance, it may neither continue the motion autonomously, nor return to the unperturbed position (there is a continuum of stable states). A cylinder on a flat smooth surface is stable in one dimension and neutrally stable in the other.

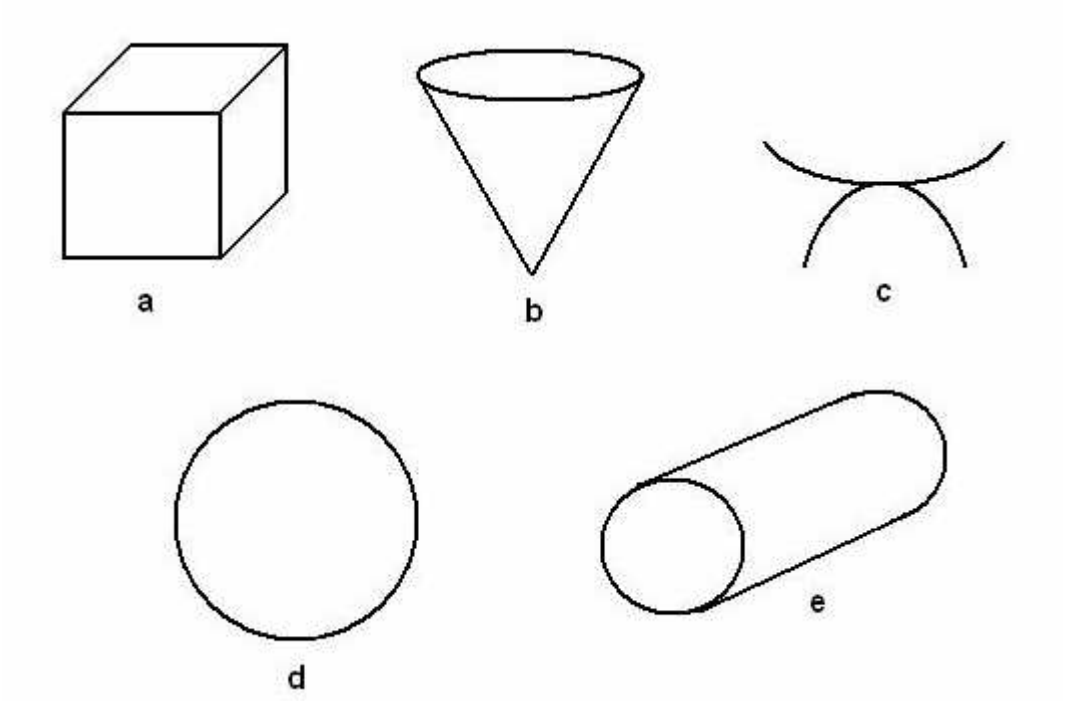
Four stable states for a given antigen

The immune system has memory; we do not get measles twice. "Memory" in a complex system means that the state of the system depends on the history of the system. For a system to have memory, it must have multiple stable states. The state "previously exposed to measles" is clearly distinct from the state "not previously exposed to measles". We call these two states the "immune" state for measles and the "virgin" (unexposed) state for measles respectively. A third state, that can be induced for many antigens, is a tolerant or unresponsive state. There are various forms of antigen-specific tolerance. We will be particularly interested in a tolerant state called the "suppressed" state. We will see that another unresponsive state, called the “anti-immune state” also emerges in the symmetrical network theory.

The postulate of symmetry as applied above to stimulation, inhibition and killing leads to the set of four stable states for a given antigen as shown in Figure 10-2. The stability of these states will be made plausible at a descriptive level, then the plausibility will be reinforced by a mathematical model of the interactions.

In the virgin state there is assumed to be a sub-threshold (not significant) amount of mutual stimulation of positive and negative cells of a given specificity. The system is assumed instead to be kept in balance by mutual killing between clones of complementary specificities. The mathematical model (below) implicates IgM antibodies rather than IgG antibodies as being important in the virgin state. The level of IgM antibody in germ-free mice (mice in the virgin state for most specificities) is not much less than in normal mice,

Figure 10-1. Various types of stability. Case a: A block on a flat surface is an "attractor" since it is stable with respect to small disturbances in two different directions at right angle to each other (x and y axes). Case b: a cone "balanced" on its point is unstable with respect to small perturbations in both the x and y directions; it is at a "repellor". Case c is a "saddle point"; it is stable with respect to small perturbations in one direction, and unstable with respect to perturbations in the other direction. Case d: a sphere is neutrally stable in both the x and y directions; perturbations neither grow nor revert to the original location. Case e: a cylinder is neutrally stable in one direction and stable in the second.



as shown in Figure 10-3,[112] so that even when the system has not been perturbed by external influences, there is a significant amount of IgM synthesis and secretion. This is in contrast to IgG, which is at a much lower level in

[112] Data assembled from measurements by S. Natsuume-Sakai, K. Motonishi and S. Migita (1977) Quantitative estimations of five classes of immunoglobulin in inbred mouse strains. Immunology, 32, 861-866.

germ free mice than in normal mice, as shown in Figure 10-4. Killing of positive cells by negative IgM antibodies and vice versa is assumed in the theory to be important in keeping the clones at low levels in the virgin state. The half-life of virgin B cells has been measured to be about one week[113]. This would seem to be metabolically wasteful in the context of a non-network view of the system, but it makes sense in the context of the symmetrical network theory, in which the virgin state is interpreted as involving a balance between non-specific influx of cells into the system and active killing by cells with the complementary specificity.

In the suppressed stable state there are elevated levels of both positive and negative T cells, and their mutual stimulation leads to a significant level of the corresponding specific T cell factors. Since these factors are monovalent, they block rather than cross-link the complementary receptors. Negative factors block positive receptors and positive factors block negative receptors. In this suppressed state there may or may not be elevated levels of specific B cells. If they are present their receptors are blocked. Tolerance to self antigens could be a consequence of an animal being in this type of suppressed state for self antigens. However, the persistent presence of a self antigen breaks the symmetry between plus and minus clones for that specificity, so the levels of plus and minus T cells specific for a self antigen are not necessarily expected to be equal.

The immune state is characterized by elevated levels of positive cells and depleted levels of negative cells. Antibodies from B cells stabilize this state by killing negative cells, thus isolating positive clones from the rest of the network. With the mathematical model we find that IgG rather than IgM is important in the immune state. This correlates well with the experimental finding that an IgM response alone is accompanied by no memory, while if there is an IgG response there is typically also memory.

The fourth stable state of Figure 10-2 is called the "anti-immune state", and is simply the converse situation to that of the immune state. It has elevated levels of negative clones and depleted levels of positive cells. Such a state could be induced by an immune response to a "positive" V region.

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113 C. J. Elson, K. F. Jablonska and R. B. Taylor (1976) Functional half-life of virgin and primed B lymphocytes. Eur. J. Immunol. 6, 634-638.

Figure 10-2. Four stable states (all attractors) for the system are the virgin state, the suppressed state, the immune state and the anti-immune state. In this model the important idiotypic interactions include killing by IgM (in the virgin state), killing by IgG (in the immune and anti-immune states) and inhibition by specific T cell factors (in the suppressed state). The population sizes of antigen-specific and antiidiotypic T cell populations are denoted T+ and T-, and similarly antigen-specific and antiidiotypic B cells are denoted B+ and B- respectively. Cross-hatched bars denote IgG antibodies, black bars denote IgM antibodies and diagonally striped bars denote specific T cell factors.

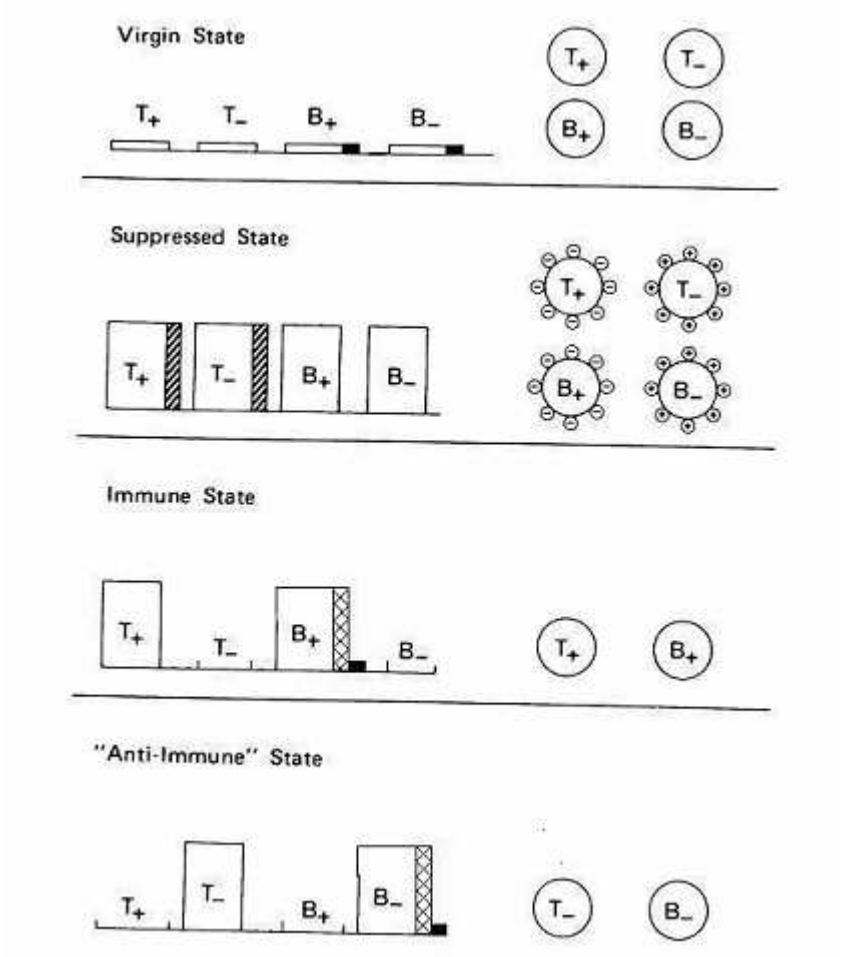


Figure 10-3. Level of IgM antibodies in normal and germ-free mice of three strains at the age of 2 months. Data from S. Natsuume-Sakai, K. Motonishi and S. Migita (1977) Quantitative estimations of five classes of immunoglobulin in inbred mouse strains. Immunology, 32, 861-866. Reproduced from G. W. Hoffmann (1979) in Lecture Notes in Biomathematics vol. 32, C. Bruni, G. Doria, G. Koch and R. Strom, Eds, Springer-Verlag, pp. 239-257.

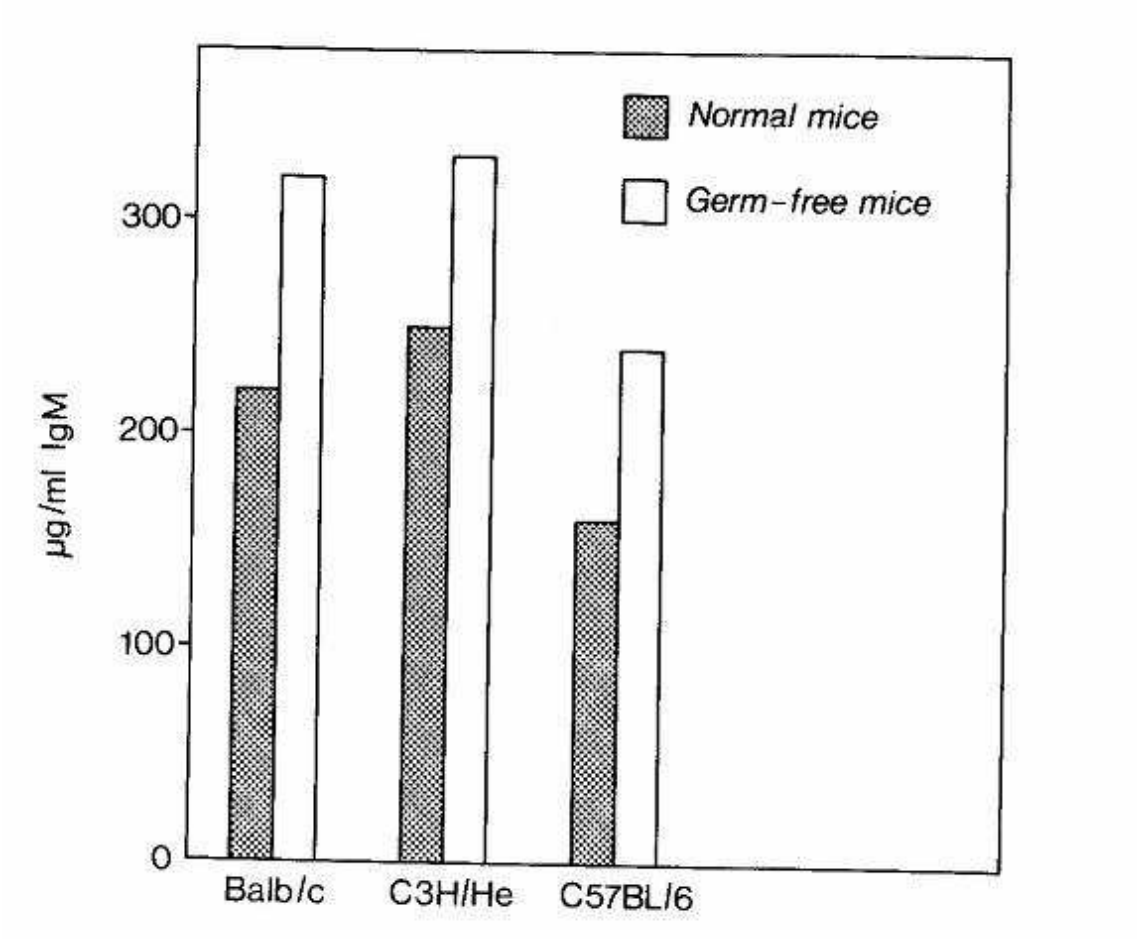
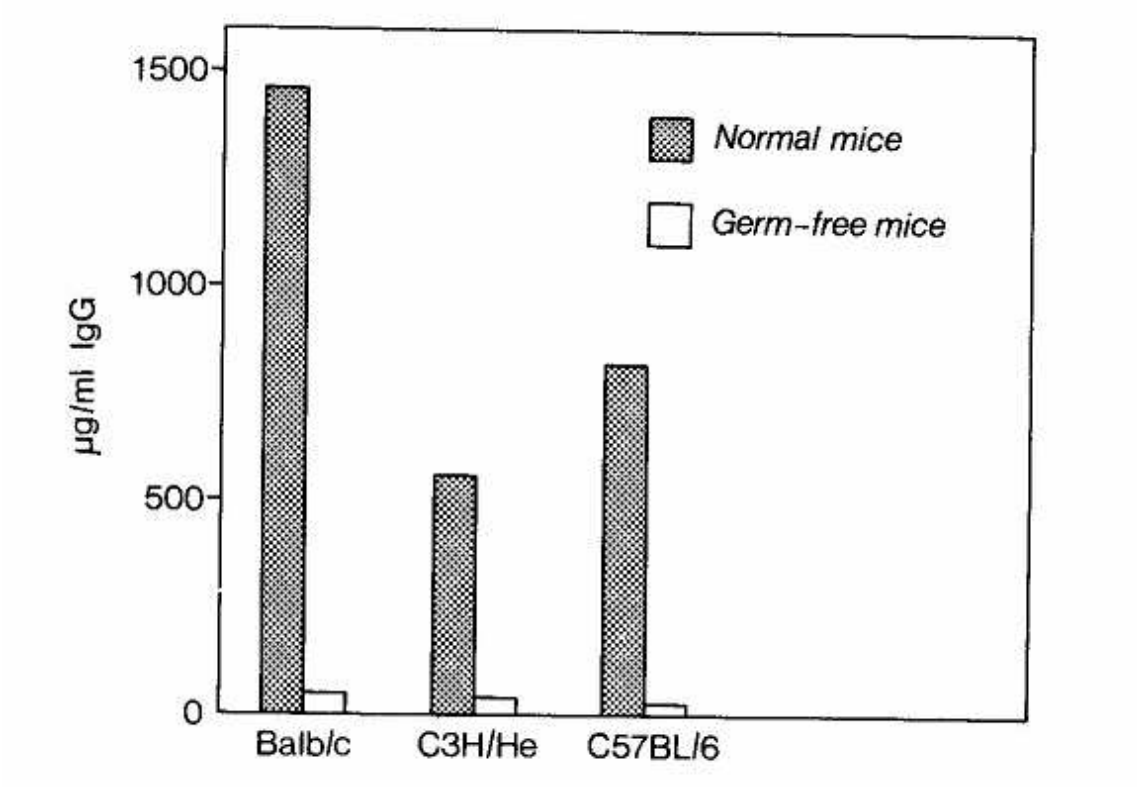


Figure 10-4. Level of IgG antibodies (sum of IgG_1, IgG_{2a} and IgG_{2b}) in normal and germ-free mice of three strains at the age of 2 months. Data compiled from S. Natsuume-Sakai, K. Motonishi and S. Migita (1977) Quantitative estimations of five classes of immunoglobulin in inbred mouse strains. Immunology, 32, 861-866. Reproduced from G. W. Hoffmann (1979) in Lecture Notes in Biomathematics vol. 32, C. Bruni, G. Doria, G. Koch and R. Strom, Eds, Springer-Verlag, pp. 239-257.



Co-selection: not all positive and negative cells are equally selected

Co-selection is a term that I have introduced to denote the mutual positive selection of individual members from within two diverse populations, such that selection of members within each population is dependent on interaction with (recognition of) one or more member(s) within the other population.[114] Co-selection is a recurring theme in the symmetrical network theory. The suppressed state as described above is an example of the result of co-selection. A diverse population of positive T cells recognizes the antigen, and interacts with a diverse population of negative T cells. Not all the positive cells will be equally strongly selected, even if they have equal affinity for the antigen, since some will interact with many, and others will interact with few negative cells. The same is true for the negative cells. The induction of the suppressed state thus involves the strong selection of a subset of the cells within the positive population and a subset of the cells within the negative population. Those positive cells that recognize not only the antigen but also as many negative cells as possible can be expected to be most strongly selected, as will the negative cells that recognize as many positive cells as possible.

T cell dependent immune responses may involve co-selection between antigen-specific B cells and antiidiotypic T cells. Detailed studies of affinity maturation, done in the context of the co-selection concept, can be expected to prove rewarding.

Network connectivity

As shown in Figure 10-2, the antigen-specific cells interact with a large population of antiidiotypic cells in the suppressed state. We accordingly call the suppressed state a state of high network connectivity. In contrast to this, in the immune state the antiidiotypic cells have been largely eliminated, the antigen-specific cells are relatively isolated idiotypically, and we call this is a state of low connectivity. The virgin state for the case shown in Figure 10-2 has an intermediate level of connectivity. Consequently, the virgin state has the potential to switch to either the suppressed or the immune state, while it is more difficult to switch from the immune to suppressed state or vice versa. If T cells in the virgin state are heterogeneous in their connectivities, those with a lower level of connectivity would be expected to act as helpers, while those with a higher level of connectivity would function as suppressors.

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[114] G. W. Hoffmann (1994) Co-selection in immune network theory and in AIDS pathogenesis. Immunol. and Cell Biol. 72, 338-346.

A two-variable mathematical model

The properties of the equilibrium states of Figure 10-2 have been investigated using the following mathematical model, which describes the interactions of positive and negative populations and changes in their concentrations as a function of time. This mathematical model provided the first evidence that the postulated steady states can indeed be stable. Small perturbations of the system in the vicinity of each of the equilibrium states are followed by the system returning to the same equilibrium state.

The model includes only positive and negative lymphocytes and their products, and does not include the antigen. This is not meant to imply that the antigen is not important. Rather, for simplicity we are modelling at this stage only the internal dynamics of the system. The model includes stimulatory, inhibitory and killing interactions as shown in Figure 10-5.

Following the modelling style of Richter, we use just two variables to denote the sizes of two populations of cells and their specific products. We use the same variable, x_1, for positive B cells, positive T cells and positive antibodies (and likewise x_2 for negative components). This is equivalent to assuming that the concentration of positive antibodies is proportional to the concentration of positive lymphocytes, and then choosing a unit for antibody concentration such that the proportionality constant is 1.

We include the following five terms in the model:

a) a mutual stimulation term for positive and negative cells specific for a particular antigen;

b) two terms that model killing of cells;

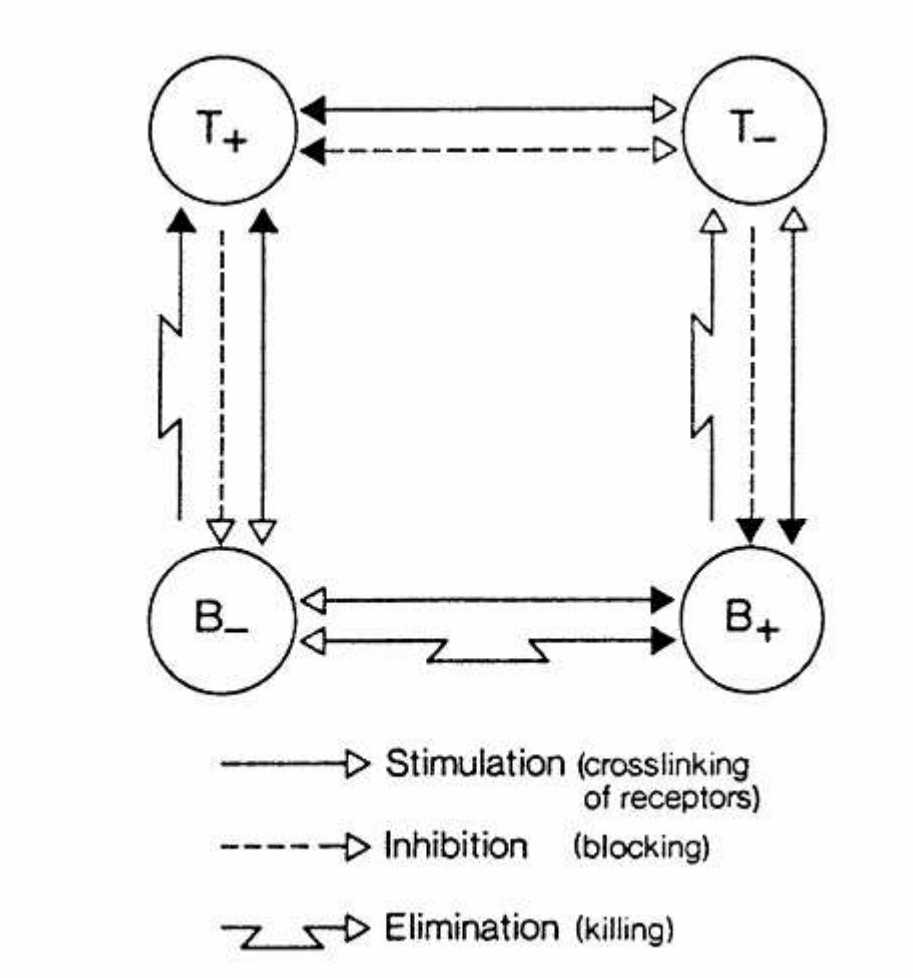
c) a term to model non-specific death (death independent of any interaction with cells of the complementary specificity) and

d) a natural birth term (also independent of any interaction with cells of the complementary specificity)

Terms a) and b) depend on V-V interactions, and can accordingly be inhibited by the V regions of specific T cell factors that can block such interactions.

The stimulation term has the form $k_1 x_1 x_2 e_1$, where k_1 is a constant, the product $x_1 x_2$ models the frequency of collisions between positive and negative cells, and e_1 is a term called the "effectivity" that models inhibition, by antigen-specific and antiidiotypic T cell factors, of stimulation of the x_2 population by the x_1 population and stimulation of the x_1 population by the x_2 population.

Figure 10-5. In a simple model of interactions in the absence of antigen, the average shape of B cell receptors that recognise the antigen (B_+) is taken to be close to the average shape of T cell receptors that recognise the antigen (T_+). Consequently the set of stimulatory, inhibitory and killing interactions that impact B_+ is taken to be the same as the set that impact T_+, and likewise for B- and T-. We model this system with just two variables, namely x_+ and x_- for idiotypic and antiidiotypic cells respectively. Reproduced from G. W. Hoffmann (1979) Lecture Notes in Biomathematics, volume 32, Systems Theory in Immunology, C. Bruni, G. Doria, G. Koch and R. Strom, Eds. Springer-Verlag, pp. 239-257.



We use the threshold function

$$e_1 = \frac{1}{1+\left(\frac{x_1 x_2}{C_1}\right)^{n_1}}$$

for the effectivity for mutual stimulation of the x_1 and x_2 populations, where n_1 and C_1 are constant parameters.

Both stimulatory and killing interactions are V-V interactions that can be blocked by the V regions of antigen-specific (positive) antiidiotypic (negative) T cell factors, that are complementary to either party of a V-V interaction. In the mathematical model we assume that the concentration of T cell factors of a given specificity is proportional to both the concentration of cells of that specificity and the concentration of cells with complementarity to that population, since the latter stimulate the first population to produce the factors. The concentration of both positive and negative specific T cell factors is accordingly assumed to be proportional to the frequency of collisions between positive and negative cells, that is, proportional to the product $x_1 x_2$. The inhibiting effect of these factors is to block and hence switch off the stimulatory and killing interactions described above. The terms for stimulation and killing therefore all include multiplicative terms that are threshold functions, and each rapidly approaches zero when $x_1 x_2$ goes much past a constant level that is characteristic of the particular process being inhibited. It is reasonable and is assumed that the different killing processes and stimulation are inhibited by different amounts of specific T cell factors, so that there are different thresholds for switching off the different processes.

For example, the effectivity (above) is a threshold term with a sharpness that depends on the parameter n_1. The larger the value of n_1, the sharper the threshold. The value of e_1 approaches 1 for $x_1 x_2 \ll C_1$ (no inhibition) and approaches 0 for $x_1 x_2 \gg C_1$ (complete inhibition).

There are four main mechanisms of killing with different concentration dependences, and our model includes two terms to take account of these. Two of the mechanisms involve antibody mediated killing, and we model this by firstly making the simplification that the amount of each specific antibody is proportional to the number of cells producing it, namely x_1 or x_2. This is in contrast to the antigen-specific T cell factors, where we assume that the amount of factor produced is proportional to $x_1 x_2$.

Two of the four killing mechanisms can be combined because they both give rise to a linear dependence of the rate of killing on the concentration of complementary cells, namely killing by IgM antibodies and killing by cytotoxic T cells. In the case of IgM, the linear dependence follows from the fact that a single IgM molecule, for example an IgM antibody with "positive" specificity, together with the set of serum proteins called complement, is able to kill a cell with "negative" specificity (Figure 2-5). Similarly, a positive cell can be killed by a single negative cytotoxic T cell, and vice versa, again giving rise to a linear concentration dependence. The killing is also linear in the concentration of cells being killed, reflecting mass action. This killing involves a specific V-V interaction, so like the stimulatory interactions, it too can be inhibited by antigen-specific and antiidiotypic T cell factors. The complete linear killing term is therefore $-k_2 x_1 x_2 e_2$, where k_2 is another constant and e_2 has the same structure as e_1, with constants C_2 and n_2.

Other killing mechanisms give rise to a stronger than linear dependence on concentration. Killing by IgG involves multiple IgG molecules (at least two) bound at adjacent sites on the cell surface and activating complement (Figure 2-6). Antibody-dependent cellular cytotoxicity ("ADCC") consists of cells such as macrophages engulfing target cells (say negative cells) that are specifically labelled by specific positive antibodies (Figure 2-7). IgG plus complement mediated killing and ADCC are accordingly both modelled by a term that is quadratic in the concentration of the cells that are doing the killing. We again have inhibition, modelled by e_3 with a form analogous to e_1 and e_2, and the quadratic killing term has the form $-k_3 x_1 x_2^2 e_1$ (for killing of positive cells) and $-k_3 x_1^2 x_2 e_3$ (for killing of negative cells).

The final two terms are a source term and a natural death term. It is assumed that positive and negative cells are generated at a constant rate, independent of the concentration of cells already present in the system. This is modeled by an additive constant S in the differential equation. It is also assumed that, even in the absence of any interactions with cells of the complementary specificity, cells have a finite lifespan. This is modelled by the terms $-k_4 x_1$ and $-k_4 x_2$ in the differential equations for x_1 and x_2 respectively. This is independent of the concentration of cells of the complementary specificity. Neither the source term nor the natural death process involves V-V interactions, and hence neither is inhibitable by specific T cell factors.

These ideas lead to the following two-variable model:[115]

$$\frac{dx_1}{dt} = S + k_1 x_1 x_2 e_1 - k_2 x_1 x_2 e_2 - k_3 x_1 x_2^{\,2} e_2 - k_4 x_1$$

$$\frac{dx_2}{dt} = S + k_1 x_1 x_2 e_1 - k_2 x_1 x_2 e_2 - k_3 x_1^{\,2} x_2 e_2 - k_4 x_2 \qquad (10.1a)$$

with

$$e_i = \frac{1}{1 + \left(\frac{x_1 x_2}{C_i} \right)^{n_i}} \qquad i = 1 \text{ to } 3 \qquad (10.1b)$$

The most obvious method for studying the model is to assign some values to the constants, then integrate the equations using a computer. The dynamics can be plotted in the x_1 / x_2 phase plane, and the locations of any attractors, repellors or saddle points determined. This approach quickly led to the conclusion that, for an appropriate choice of the constants, four stable states (attractors) corresponding to the virgin, immune, anti-immune and suppressed states as described above can indeed exist, as shown for one set of parameters in Figure 10-6.

Much or our analysis has been done on a simplified version of this model, namely one in which the thresholds are made sharp, and we do not include the mutual stimulation term $k_1 x_1 x_2 e_1$. Mutual stimulation of positive and negative clones is then nevertheless still implicit in the form of the effectivity threshold functions. The model works fine without the $k_1 x_1 x_2 e_1$ term, and it makes sense to at least initially keep it as simple as possible. This yields the simpler system

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115 G. W. Hoffmann (1979) "A mathematical model of the stable states of a network theory of self regulation" in Lecture Notes in Biomathematics vol. 32 (C. Bruni, G. Doria, G. Koch, and R. Strom, Eds.) 239-257.

$$\frac{dx_1}{dt} = S - k_2 x_1 x_2 e_2 - k_3 x_1 x_2^{\,2} e_3 - k_4 x_1$$

$$\frac{dx_2}{dt} = S - k_2 x_1 x_2 e_2 - k_3 x_1^{\,2} x_2 e_3 - k_4 x_2 \qquad (10.2a)$$

with

$$e_q = 1 \text{ when } x_1 x_2 < C_q$$
and $q = 2, 3$ (10.2b)
$$e_q = 0 \text{ when } x_1 x_2 > C_q$$

The dynamics of this system for another set of parameters is shown in Figure 10-7. In this particular case we have a different region of parameter space altogether from that of Figure 10-6, (see the constraints on parameters below, equations 10.15a and 10.15b), and the pattern of trajectories looks quite different. The phase plane plot nevertheless shows that the population levels x_1 and x_2 always converge on one of four attractors, that can be identified with the virgin state, the immune state, the suppressed state and the anti-immune state.

A network rational for the existence of both IgM and IgG

The system can have the desired four steady stable states without the inclusion of the clonal expansion due to mutual stimulation term of specificity 1 (plus) clones by specificity 2 (minus) clones. Figure 10-7 is an example of one such case; it has $k_1 = 0$. Mutual stimulation of plus and minus cells is nevertheless implicitly modeled, in that the inhibition terms contain the product $x_1 x_2$, reflecting the idea that the amount of inhibition depends on the frequency of collisions between cells with plus and minus specificities. On the other hand, both a nonlinear killing term and a linear killing term are needed for the model to work. Figure 10-8 and Figure 10-9 illustrate typical dynamics when one or the other of these two terms is missing. Without the linear killing term the virgin state becomes a saddle point (unstable with respect to changes in two of four directions), and without the quadratic term the immune state and the anti-immune state no longer exist. There is typically a switch from making IgM to making IgG during an immune response that has memory, and the roles of the two classes are thus in good agreement with the roles they have in this model. Furthermore, germ free mice, that have undergone no immune responses have

Figure 10-6. Phase plane dynamics for the system 10.1 with the parameters that satisfy the inequalities 10.15a (parameter space "type 1"), namely: $k_1 = 0.1$, $k_2 = 1$, $k_3 = 1$, $k_4 = 0.01$, $S = 1$, $C_1 = 100$, $C_2 = 9$, $C_3 = 0.09$, $n_1 = 1$, $n_2 = 2$, $n_3 = 2$. The variable x_+ is synonymous with x_1 of the system 10.1, and x_- is synonymous with x_2. Here the virgin state (VS) is at $x_1 = x_2 \approx 1$, the suppressed state (SS) is at $x_1 = x_2 \approx 1000$, the immune state (IS) is at $x_1 \approx 100$, $x_2 \approx 10^{-4}$, and the anti-immune state (AIS) is at $x_2 \approx 100$, $x_1 \approx 10^{-4}$. Reproduced from G. W. Hoffmann (1979) in Lecture Notes in Biomathematics vol. 32 (C. Bruni, G. Doria, G. Koch, and R. Strom, Eds.) 239-257.

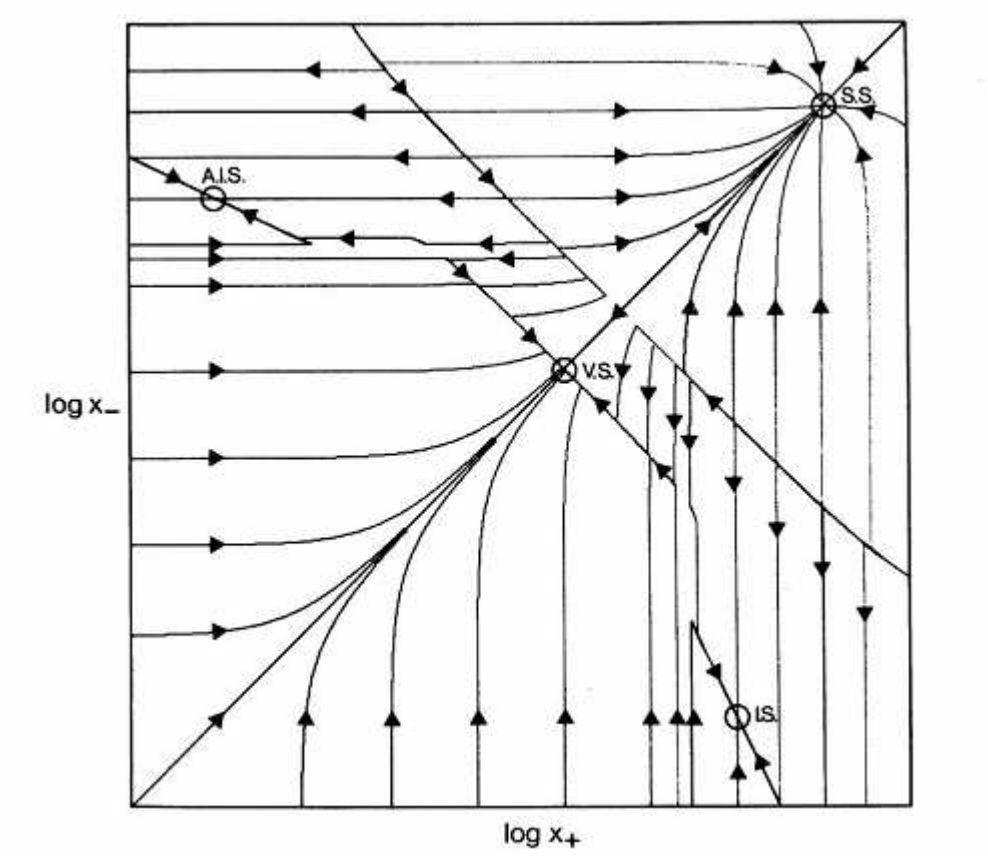
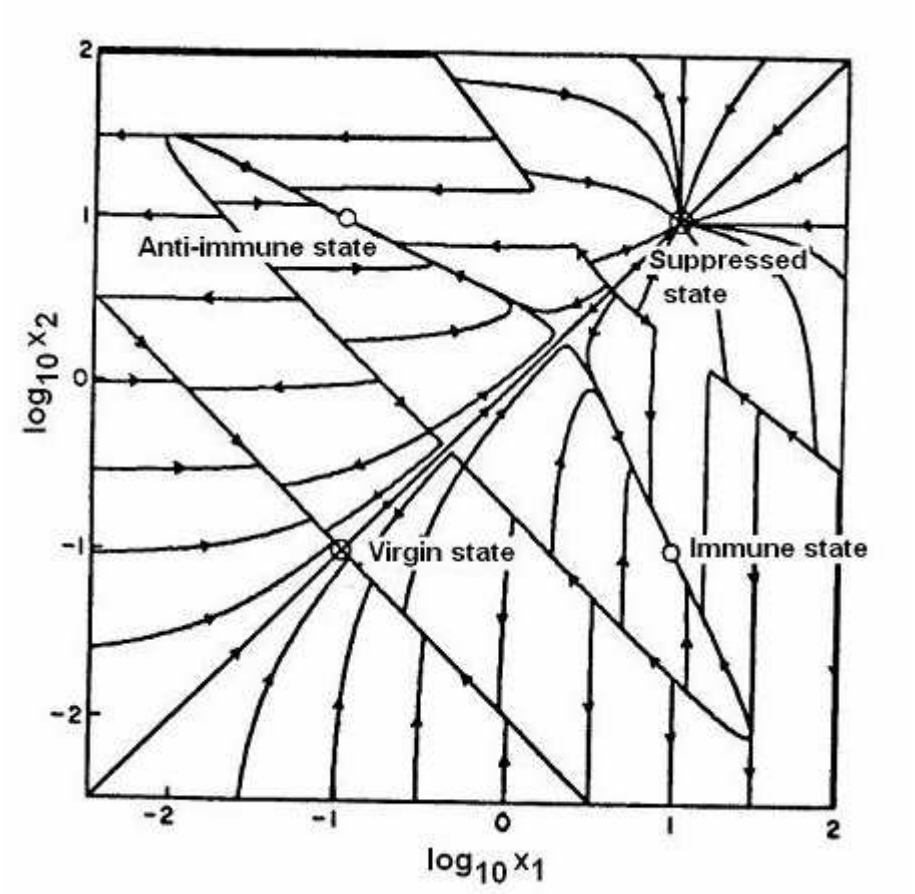


Figure 10-7. An example of phase plane dynamics with parameters that satisfy the inequalities 10.15b (parameter space "type 2"). Parameters: S = 1, k_1 = 0, k_2 = 100, k_3 = 0.1, k_4 = 0.1, C_2 = 0.1, C_3 = 10, n_2 = 5, n_3 = 5. This illustrates that a minimal model does not need to include the term modelling proliferation due to mutual stimulation of x_1 and x_2 clones, for the four stable states to exist. Mutual stimulation between x_1 clones and x_2 clones is nevertheless implicit in the $x_1\ x_2$ product that is present in the terms that model inhibition of IgM and IgG killing by antigen-specific T cell factors. Adapted from N. Gunther and G. W. Hoffmann (1982) J. theoret. Biol. 94, 815-855.



approximately normal levels of IgM, but very low levels of IgG.[112] All of these phenomena are consistent with IgM being important for the stability of the virgin state, and IgG being important for immunity and memory, as is the case in the model.

These ideas are reinforced by closer inspection of the mathematical model. Without the nonlinear killing term, the model yields

$$\frac{dx_1}{dt} - \frac{dx_2}{dt} = -k_4(x_1 - x_2) \tag{10.3}$$

for which the only steady states occur when $x_1 = x_2$. The immune state, in which the symmetry between x_1 and x_2 is broken, is dependent in this model on a non-linear killing term such as that provided by IgG.

Without the linear killing term, four steady states can still exist; we could then perhaps choose parameters such that the virgin state is a balance between IgG killing and non-specific influx for both x_1 and x_2 populations thus

$$\begin{aligned} \frac{dx_1}{dt} &\approx S - k_3 x_1 x_2^2 \\ \frac{dx_2}{dt} &\approx S - k_3 x_2 x_1^2 \end{aligned} \tag{10.4}$$

We would then have $x_1 = x_2 \approx \sqrt[3]{S/k_3}$ in this putative virgin state. But is this steady state stable? Would a small perturbation of it result in the system returning to the steady state, or would the perturbation grow with time? To answer this we need a method for differentiating between stability and instability in such systems.

Figure 10-8. An example of the phase plane dynamics of system 10.1 with the IgM term and no IgG term. Parameters as for Figure 10-6 except that $k_3 = 0$. Reproduced from G. W. Hoffmann (1979) in Lecture Notes in Biomathematics vol. 32 (C. Bruni, G. Doria, G. Koch, and R. Strom, Eds.) 239-257.

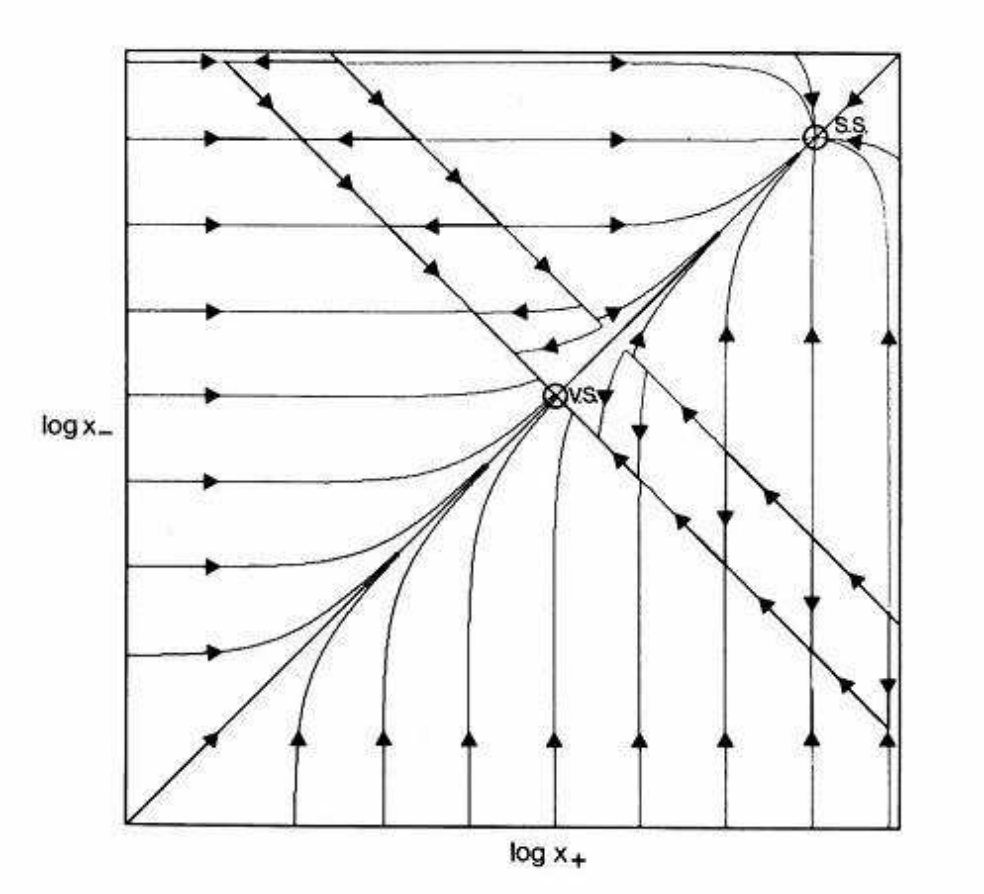
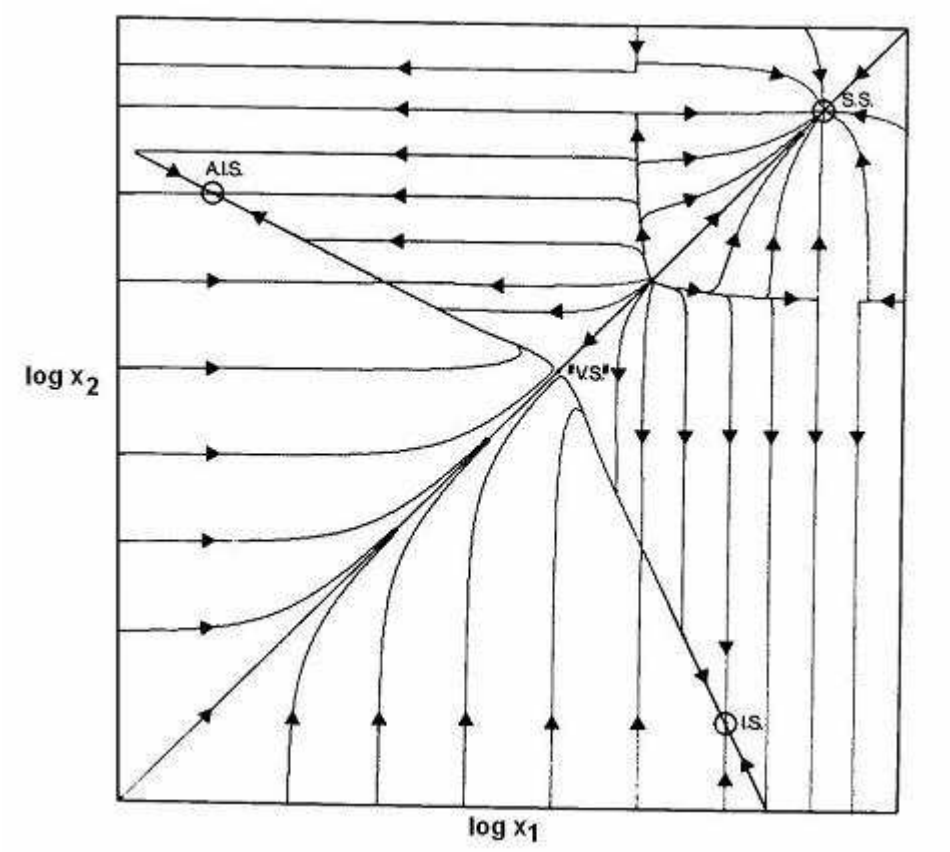


Figure 10-9. An example of the phase plane dynamics of system 10.1 including the IgG term and no IgM term. Parameters as for Figure 10-6 except that k_2 = 0 and C_3 = 9. In this case the "virgin state" is unstable (a saddle point), and there is a repellor between that point and the suppressed state. Reproduced from G. W. Hoffmann (1979) in Lecture Notes in Biomathematics vol. 32 (C. Bruni, G. Doria, G. Koch, and R. Strom, Eds.) 239-257.



The eigenvalue method of stability analysis

The eigenvalue method is a powerful tool for determining whether a steady state is stable or unstable, and if it is stable, whether it is a repellor or a node.

If we have a system that is modeled by N differential equations, with a steady state $x_1, x_2 \ldots\ldots x_N$, which we abbreviate as $\mathbf{x}_0$, we can analyze the stability of the system at the steady state by calculating a set of numbers called the eigenvalues ("λ_i") of the system. The eigenvalues are determined as follows. The system of differential equations may be written

$$\frac{d\mathbf{x}}{dt} = \mathbf{F}(\mathbf{x})$$

where $\mathbf{x}$ is a vector $(x_1, x_2 \ldots, x_N)$ and $\mathbf{F}$ is a set of functions $F_1, F_2 \ldots, F_N$ of $\mathbf{x}$. Let $\mathbf{A}$ be the matrix with elements $A_{ij} = \frac{\partial F_i}{\partial x_j}$ evaluated at a steady state point $\mathbf{x_0}$, which is a point defined by $\frac{d\mathbf{x}}{dt} = 0$. $\mathbf{A}$ is called the Jacobian matrix of the system at $\mathbf{x_0}$. The stability of the system at $\mathbf{x_0}$ is determined by the eigenvalues λ_i ($i = 1, N$) of $\mathbf{A}$. If $\mathbf{I}$ is the unit matrix (with elements $I_{ij} = 1$ for $i = j$ and $I_{ij} = 0$ for $i \neq j$), the eigenvalues λ_i are the solutions of $\det(\mathbf{A} - \lambda\mathbf{I}) = 0$. Points with one or more positive eigenvalues are unstable, while points for which eigenvalues are negative are stable. Eigenvalues equal to zero correspond to neutral stability. Complex eigenvalues are indicative of oscillatory behaviour.

Back to the need for both IgM and IgG

In the case of a two-variable system ($N = 2$) equation (10.5) is shorthand for

$$\frac{dx_1}{dt} = F_1(x_1, x_2)$$
$$\frac{dx_2}{dt} = F_2(x_1, x_2) \qquad (10.6)$$

We can simplify the system (10.4), a putative model for the virgin state with only IgG, by choosing time and concentration units such that $k_3 = S = 1$, without changing the qualitative nature of the dynamics. So we investigate the stability of the system

$$\frac{dx_1}{dt} = F_1(x_1, x_2) \approx 1 - x_1 {x_2}^2$$
$$\frac{dx_2}{dt} = F_2(x_1, x_2) \approx 1 - {x_1}^2 x_2 \qquad (10.7)$$

The steady state of the system (10.7) is at $x_1 = x_2 = 1$. The elements of the Jacobian matrix at the steady state are then

$$A_{11} = \frac{\partial F_1}{\partial x_1} \approx -{x_2}^2 = -1$$

$$A_{12} = \frac{\partial F_1}{\partial x_2} \approx -2x_1 x_2 = -2$$

$$A_{21} = \frac{\partial F_2}{\partial x_1} \approx -2x_1 x_2 = -2$$

$$A_{22} = \frac{\partial F_2}{\partial x_2} \approx -{x_1}^2 = -1$$

and the Jacobian matrix is

$$\boldsymbol{A} = \begin{pmatrix} -1 & -2 \\ -2 & -1 \end{pmatrix}$$

The two eigenvalues are obtained thus:

$$\begin{vmatrix} -1-\lambda & -2 \\ -2 & -1-\lambda \end{vmatrix} = 0$$

$$(-1-\lambda)^2 = 4$$

$$-1-\lambda = \pm 2$$

and

$$\lambda_1 = 1, \quad \lambda_2 = -3$$

So we find that one of the eigenvalues is negative and one is positive. Such a point is stable with respect to motion in one direction, but unstable with respect to motion in another direction. Such a point is called a saddle point, and due to the instability in one direction it is unstable overall. Hence it appears that we need both linear and nonlinear killing terms, such as those provided by IgM and IgG for the system to work. This result provides a rationale for the existence of these two major antibody isotypes.

As a second example of the application of this method, we now analyse the stability of the immune state for the case of IgG and no IgM. The terms that are postulated to be important at the immune state are $-k_4 x_1 + S$ (for x_1) and $-k_3 x_1^2 x_2 e_3 + S$ (for x_2). Parameters are chosen such that e_3 is approximately equal to one. The equations then have the approximate form

$$\frac{dx_1}{dt} = F_1(x_1, x_2) \approx k_4 x_1 + S = 0$$

$$\frac{dx_2}{dt} = F_2(x_1, x_2) \approx -k_3 x_1^2 x_2 e_3 + S$$

Then we have

$$A_{11} \approx -k_4$$

$$A_{12} \approx 0$$

$$A_{21} \approx -2k_3 x_1 x_2$$

$$A_{22} \approx -k_3 x_1^2$$

and the eigenvalue equation is

$$\begin{vmatrix} -k_4 - \lambda & 0 \\ -2k_3 x_1 x_2 & -k_3 x_1^{\,2} - \lambda \end{vmatrix} = 0$$

Then

$$(-k_4 - \lambda)(-k_3 x_1^{\,2} - \lambda) = 0$$

so that

$$\lambda_1 = -k_4 \text{ and } \lambda_2 = -k_3 x_1^{\,2}$$

Both eigenvalues are negative so this steady state is stable. This is consistent with the phase plane trajectories of Figure 10-9.

Oscillations

The inclusion of the mutual stimulation term, $k_1 x_1 x_2 e_1$ in the model leads to the possibility of oscillations in x_1 and x_2. If this stimulation term is large compared with the influx term S, and the efficacies are given approximately by $e_1 = e_3 = 1$ and $e_2 = 0$, the system reduces approximately to

$$\frac{dx_1}{dt} = F_1(x_1, x_2) \approx k_1 x_1 x_2 - k_4 x_1$$

$$\frac{dx_2}{dt} = F_2(x_1, x_2) \approx k_1 x_1 x_2 - k_3 x_1^{\,2} x_2$$

There is then a steady state at

$$x_1 \approx k_1 / k_3$$

$$x_2 \approx k_4 / k_1$$

The Jacobian matrix at this steady state is

$$\mathbf{A} = \begin{pmatrix} 0 & k_1^2/k_3 \\ -k_4 & 0 \end{pmatrix}$$

The eigenvalues of this matrix are $\pm i\sqrt{k_4 k_1^2/k_3}$, where $i = \sqrt{-1}$. Imaginary eigenvalues are characteristic of oscillatory behaviour. Phase plane dynamics with such oscillations are shown in Figure 10-10. The circular motion corresponds to oscillations in both x_1 and x_2 that are out of phase with each other. This result is of interest because damped oscillations in the number of antigen-specific plaque forming cells,[116] or in the concentration of specific antibodies[117] have been observed in immune responses. It has not been shown (and is not necessarily the case) that the oscillations observed experimentally are due to oscillations of idiotypes and antiidiotypes that are out of phase with each other. It is nevertheless of interest that the model can exhibit oscillatory behaviour.

The system (10.1) has also been analyzed in greater detail by Gunther and Hoffmann[118] using a theorem of Poincaré together with the fact that the differential equation can be written in the form

$$\frac{dx_1}{dt} = Ax_2 + Bx_1 + C$$

$$\frac{dx_2}{dt} = Ax_1 + Bx_2 + C$$

where A, B and C are functions of $p = x_1 x_2$ only. This analysis led to both qualitative and quantitative information about the parameter space. For example, it revealed that the mutual stimulation term (k_1 term) is not necessary

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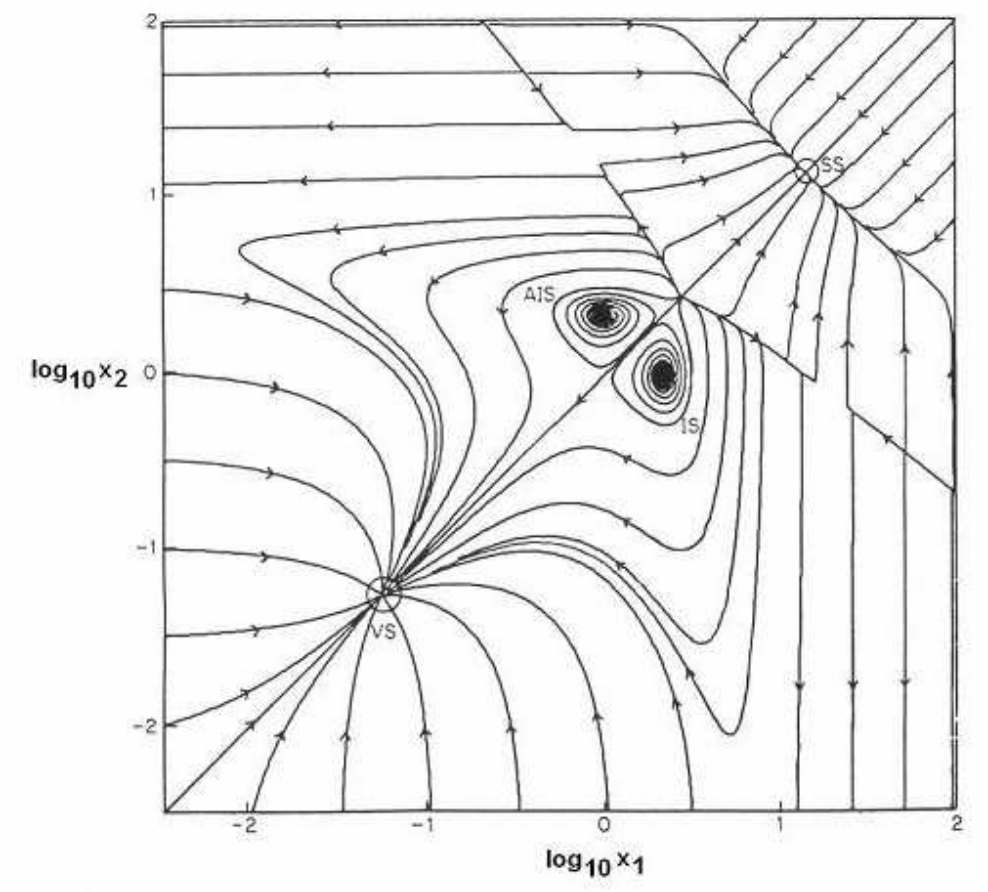
116 C. G. Romball and W. O. Weigle (1973) A cyclical appearance of antibody-producing cells after a single injection of of serum protein antigen. J. Exp. Med. 138, 1426-1442.

117 J. W. Kimball (1972) Maturation of the immune response to type three pneumococcal polysaccharide. Immunochemistry 9, 1169-1184.

118 N. Gunther and G. W. Hoffmann (1982) "Qualitative Dynamics of a Network Model of Regulation of the Immune System: A Rationale for the IgM to IgG Switch", J. theoret. Biol. 94, 815-855.

Figure 10-10. An example of phase plane dynamics of the system 10.1 with oscillations as the system approaches the immune state. Parameter values are $k_1 = 3$, $k_2 = 0$, $k_3 = 1$, $k_4 = 2$, $S = 0.1$, $C_1 = 100$, $C_3 = 10$, $n_1 = 5$, $n_3 = 5$, C_2 and n_2 arbitrary. From N. Gunther and G. W. Hoffmann (1982) J. theoret. Biol. 94, 815-855.



in order for the model to work, that is, for the model to yield the four stable states. Figure 10-7 shows an example of the phase plane dynamics with $k_1 = 0$. The analysis furthermore led to the conclusion that there are two regions of parameter space that each exhibit at least four stable steady states. Providing the thresholds are made sharp, these regions correspond to the following sets of inequalities in the parameter space:

$$\frac{k_4}{k_3} < C_3 < \frac{S}{k_2} < C_2 < \left[C_1 \; or \left(\frac{S}{k_4} \right)^2 \right]$$

and

$$\frac{S}{k_2} < C_2 < \frac{k_4}{k_3} < C_3 < \left[C_1 \; or \left(\frac{S}{k_4} \right)^2 \right]$$

These two regions are characterized by different phase plane dynamics. Figure 10-6 has parameters that satisfy the condition 10.15a, while Figure 10-7 has parameters that satisfy the condition 10.15b.

The dimensions of all the terms in the inequalities are [concentration]^2, the same dimensions as p. On the log-log plots of Figures 10-4 to 10-8 diagonal straight lines at right angles to the diagonal line log x_1 = log x_2 are lines with a constant value of p.

The difference between the two regions of parameter space is that for (10.15a) the immune and anti-immune states are situated at a lower value of p than the virgin state, while for (10.15b) the converse is the case. In both cases we have $S / k_2 < C_2$, an inequality that is particularly relevant to the stability of the virgin state, and $k_4 / k_3 < C_3$, an inequality relevant to the immune and anti-immune states.

Remarkably, neither of the sets of inequalities imposes any restriction on the value of k_1. Hence we can have k_1 being zero or any positive number, and still have a set of four stable steady states.

This analysis provided a more detailed rationale for the existence of both IgM and IgG, with IgM being important for the virgin state, and IgG being important for the immune state.

It is surprising how well this mathematical model works. There are important differences between T cells and B cells that we have not incorporated into the model, since we do not even have separate variables for T cells and B cells. In particular, the fact that T cells respond to the presence of much lower amounts of antigen than B cells is undoubtedly important, and a more complete model would incorporate that aspect. Because they are so much more sensitive to antigen, T cells must be the prime regulating elements in the system, and it would be nice to know more about the steady state that is reached for T cells in the immune state. A fuller description would include the number of antigen-specific and antiidiotypic T cells that are present as a function of affinity for the antigen, of affinity for other T cell idiotypes and for B cell idiotypes, including at least both IgM and IgG idiotypes. T cells use a different set of V genes than B cells, but they nevertheless manage to cover the spectrum of all possible antigens. If we attempt to take all this into account, we find that the mathematical model quickly becomes unmanageably complex, and we are therefore left with having to develop what is doubtless a highly simplified picture. I suspect that in reality at least antiidiotypic T cells play a larger role in the immune state than they do in this simple model, where we have assumed that both antiidiotypic B and T cells are essentially eliminated in the immune state.

The high turnover rate of lymphocytes

There is a high turnover rate of lymphocytes in the immune system. About 50-60% of lymphocytes are replaced every 2-3 days, even if the immune system is not obviously doing anything, that is, it is not responding to a foreign antigen. In the context of a simple clonal selection view of the immune system, this is a paradox. It would appear to be a waste, since the new lymphocytes are rapidly replacing perfectly good, recently produced lymphocytes. This is happening at a high metabolic cost, and is similar to the situation in the brain, that also uses a significant amount of energy to maintain neural memory. The resolution of the paradox comes from the necessity to have death in the system (and hence death terms in our equations), in order to achieve multiple steady states and immune memory. The active killing of lymphocytes leads to the high turnover rate at steady population levels. Our steady states are thus unlike chemical equilibrium states, that do not require energy to be maintained.

On tolerance and suppression

Tolerance is a broadly used term that is synonymous with unresponsiveness, which in many cases occurs without suppression being demonstrable. The word suppression is usually reserved for situations in which tolerant cells actively prevent a response by unprimed cells in mixing

experiments. The question arises of whether these are two distinct phenomena, or whether the same underlying mechanisms can account for them both. In our diagrams showing the dynamics of the two variable model, there is a suppressed state, and if we were to mix a system that is in this state with a system consisting of unprimed cells, we would have a system that is situated somewhere along the line between the virgin state and the suppressed state. The trajectory of the resulting system could then be either towards the virgin state or towards the suppressed state, depending on the starting position relative to the repellor that lies between them. The cells themselves are not "responsive" or "suppressed", rather the position of the populations on the phase plane determines the outcome of particular mixing experiments.

The mathematical model suggests the following experiment. Generate the tolerant (suppressed) state for an antigen in a group of animals. Take pooled spleen cells from these animals, and likewise pool spleen cells from a group of naive animals of the same strain. Irradiate a third group of animals, and reconstitute them with a fixed total number of cells from the two groups of animals, varying the ratio of suppressed to naive. Challenge the animals with antigen only after allowing a significant time interval for the animals to reach a stable steady state, namely either the virgin state or the suppressed state. The model predicts that responses at low ratios of suppressed to virgin cells will uniformly be the same as those seen with virgin cells alone, while those with higher ratios will make responses equal to those seen in controls with only suppressed cells. The model predicts that there will be a sharp transition from fully responsive to fully suppressed as the ratio is varied, and the mixture crosses the point on the phase plane corresponding to the repellor between the virgin and suppressed states.[119] Analogous predictions can be made for experiments with virgin and immune cells or immune and suppressed cells. (Virgin and immune cells have been known to combine to produce an unresponsive state.) In this way it should be possible to experimentally map the regions of attraction for the virgin, immune and suppressed states in the x_1 / x_2 phase plane for the antigen being used.

Switching between stable states

The emphasis in the development of the symmetric theory has been on developing an understanding of specific mechanisms and how they relate to the stability that is necessary for the system to have long term memory. As we have seen, this did not require any reference to non-specific components such as macrophages and non-specific soluble mediators, such as the interleukins. When we come to the question of how the system can switch between stable

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[119] Prediction

states, we find however that we need to invoke such components. The difficulty is that there are many such interleukins, and at least some of them appear to be produced by multiple cell types and/or interact with multiple cell types. We will incorporate non-specific factors into the model with a partly top-down approach that emphasizes simplicity and is also based on an analogy with a well-known system. The actual system is almost certainly more complex than the model we develop using this approach. We are however keeping the model as simple as possible; additional components are to be added only on an as-needed basis.

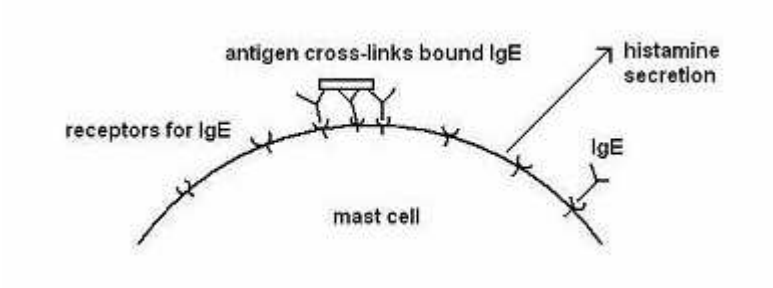
There is an enormous amount of data on non-specific factors, and our approach is a compromise between the requirement of simplicity and the requirement of having seamless support from the literature. It is expected that we can then develop insights about the way in which the universe of V-V interactions can interface with the universe of non-specific mediators. The model we present here is only a beginning in the area of non-specific interactions. I will be surprised if a simpler model with comparable scope can be found.

While a B cell can be stimulated to proliferate using something that cross-links its receptors, it typically needs an additional stimulus to make it secrete antibodies. Similarly, an antigen-stimulated T cell typically needs a second stimulus to enable it to proliferate. These additional stimuli are often called "second signals", and are believed to be important for the switching between stable steady states for specific antigens. We will present a model of how second signals are produced and received by T cells and B cells. This extends the theory significantly, in a way that is consistent with the importance that we have ascribed to the cross-linking of receptors as a central mechanism. Before presenting the model, we describe an analogous, better characterized system.

The IgE story

Mast cells and basophils are non-specific cells (no antibody or antibody-like receptors) that are involved in allergies. They secrete histamine when they are activated by an allergen (an allergy inducing substance; for example, a food that induces allergy, pollen, dust components, wasp venom). But how can mast cells and basophils be activated by allergens if they do not have receptors for the specific foreign substances? While the mast cell is a non-specific cell, it does have a receptor for the IgE class of antibodies. The constant region of IgE binds to the mast cell. If a person is immune to (say) pollen, and is makes anti-pollen antibodies of the IgE class, then his or her mast cells will have anti-pollen antibodies bound to their surfaces, and when the antigen comes along, it cross-links the IgE antibodies, which activates the mast cell, and it releases histamine (Figure 10-11).

Figure 10-11. Mechanism for the stimulation of mast cells (also basophils) to secrete histamine. IgE binds to a receptor for IgE on the mast cell surface. An antigen, which has to be at least divalent, binds to two or more of the bound IgE antibodies, cross-linking them. The IgE receptors cross the membrane, and dimers or other multimeric forms of the receptors provide a structure within the cell that triggers histamine release.



A great deal of evidence supports the cross-linking mechanism for this system, as shown in Table 10-1, which is adapted from Metzger[120]. The evidence is analogous to the data that proves B cells are stimulated by the cross-linking of Ig receptors. A univalent hapten x is unable to cause activation of mast cells sensitized with IgE that is anti-x, while multivalent x is able to do so. If a hapten x is covalently coupled to a second hapten y, the resulting $x-y$ reagent is able to activate cells sensitized with anti-x plus anti-y, but not cells sensitized with only anti-x or only anti-y. Fab fragments of antibodies with anti-IgE specificity are unable to activate cells sensitized with IgE, while IgG that is anti-IgE can activate such cells. Finally, monomeric Fc fragments obtained from IgE are unable to activate unsensitized mast cells, while such fragments in aggregated form are able to do so. All of these results are consistent with the cross-linking model for the activation of mast cells via IgE.

Additional rigorous proof comes from quantitative studies of the amount of histamine release as a function of the logarithm of bivalent antigen concentration. Experiments showed that this gives a bell-shaped curve.[121] Dembo and Goldstein of the Theoretical Biology and Biophysics Group at Los Alamos National Laboratory mathematically modelled the interaction between

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[120] H. Metzger (1977) in Receptors and Recognition, Ser. A, Vol. 4 (P. Cuatrecasas and M. F. Greaves, Eds.), pp. 75-102, Chapman and Hall, London.

[121] R. P. Siraganian, W. A. Hook and B. B. Levine (1975) Specific *in vitro* histamine release from basophils by bivalent haptens: evidence for activation by simple bridging of membrane bound antibody. Immunochem. 12, 149-157.

mast cells, IgE and antigen, and showed that such bell-shaped curves are indeed to be expected on the basis of the assumption that the amount of histamine release is proportional to the number of cross-linked bound IgE antibodies.[122] This work culminated in successful collaboration with experimentalists, that further established the case for cross-linking of the cell-bound IgE as the stimulation mechanism in this sytem.[123] A small amount of bivalent hapten results in a corresponding small amount of cross-linking. An intermediate amount of bivalent hapten results in a maximum in the amount of cross-linking and histamine release. Very large amounts of the bivalent hapten result in saturation of all the IgE binding sites. In this case the free bivalent hapten in the solution inhibits cross-linking by bivalent hapten that is bound to one arm of a bound IgE antibody. The modelling and experiments included determining the amount of inhibition of cross-linking by monovalent hapten, and again there was good agreement between the cross-linking theory and experiments.

Our model for switching between stable steady states in the symmetrical network theory involves a non-specific accessory cell in a way that is analogous to the role of the (non-specific) mast cell in this system, and provides an interpretation of low dose and high dose tolerance. Now we will turn to switching between stable states for T cells and B cells, including IgM and IgG immune responses.

Activated A cells secrete a non–specific factor that facilitates the proliferation of T cells

In order to account for switching between stable states we need to invoke non-specific accessory cells. These are also known as A cells, and they include macrophages and monocytes. When A cells are activated, one of the results is that they secrete the non-specific factor IL-1, which provides T cells with a second signal (in addition to the signal mediated via the specific T cell receptor), that enables them to proliferate.

[122] M. Dembo and B. Goldstein (1978) Theory of equilibrium binding of symmetric bivalent haptens to cell surface antibody: application to histamine release from basophils. J. Immunol. 121, 345-353.

[123] M. Dembo, B. Goldstein, A. K. Sobotka and L. M. Lichtenstein (1978) Histamine release due to bivalent penicilloyl haptens: control by the number of cross-linked IgE antibodies on the basophil plasma membrane. J. Immunol. 121, 354-358.

B cells respond to cross-linking of receptors plus a non-specific differentiation factor by becoming antibody secreting cells

It would be wasteful for B cells to be making and secreting antibodies all the time, and even the binding of antigen to a B cell's receptors with the cross-linking of the receptors does not suffice to switch it on to become an antibody secreting cell or "plasma cell". A second signal is required, and there is evidence that this is a soluble non-specific factor. Such factors were originally called T cell replacing factor (TRF) or B cell differentiation factor (BCDF). Now several interleukins have been found to have such a property, including IL-6.[124]

The role of the A cell in B cell responses is envisaged as follows. Antigen-specific T cell factors bind to the surface of A cells,[125] so the A cell presumably has a receptor for the constant part of the specific T cell factors. It is postulated that cross-linking of that receptor by the antigen via the specific T cell factor activates the A cell to secrete non-specific factors. (Figure 10-12). At least one of the non-specific factors provides B cells with a second signal, which permits the B cells to differentiate from a form that secretes very little antibody, to a plasma cell, which secretes large amounts of antibody. Non-specific factors with this property were first discovered and partly characterized by Schimpl and Wecker of Germany,[126] and by Dutton and coworkers in the U.S.A.[127] In our model, the B cells that receive a signal via their specific receptors (via cross-linking) express receptors for the non-specific factor. This helps to ensure specificity in the B cell response.

Low dose tolerance, the immune response and high dose tolerance

Incorporation of the A cell into the model in this way makes it possible to explain firstly the helper functions of T cells, and secondly the remarkable dose-response properties of the system. The postulated second signal role of the A cell derived non-specific factor automatically leads to lower and upper

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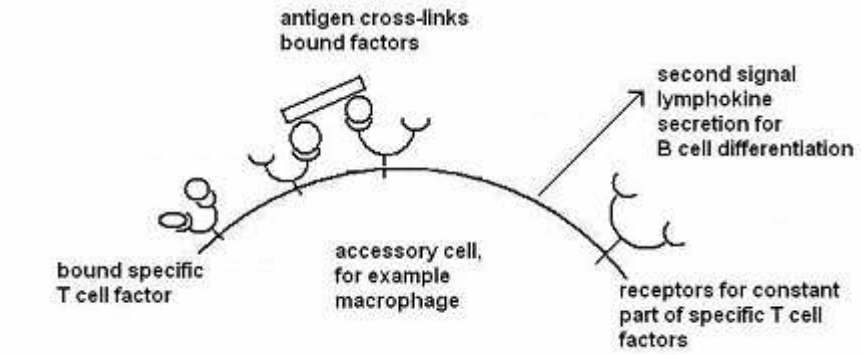
124 S. K. Durum and J. J. Oppenheim (1989) Macrophage-derived mediators: Interleukin 1, Tumor Necrosis Factor, Interleukin 6, Interferon and related cytokines. In "Fundamental Immunology, second edition" W. E. Paul, Raven Press Ltd., New York, 1989, pp. 639-661.

125 R. Evans, C. K. Grant, H. Cox, K. Steel and P. Alexander (1972) Thymus-derived lymphocytes produce an immunologically specific macrophage-arming factor. J. Exp. Med., 136, 1318-1322.

126 A. Schimpl and E. Wecker (1975) A third signal in B cell activation given by TRF. Transpl. Rev. 23, 176-188.

127 R. W. Dutton (1975) Separate signals for the initiation of proliferation and differentiation in the B cell response to antigen. Transpl. Rev. 23, 66-77.

Figure 10-12. Model for the roles of antigen, antigen-specific T cell factors and non-specific accessory cells (for example monocytes or macrophages) in producing a second signal lymphokine, that gives dividing B cells a second signal, inducing them to differentiate from dividing cells to antibody producing plasma B cells. IL-6 is such a non-specific lymphokine. The similarity with the mechanism for triggering mast cells to release histamine (Figure 10-11) is evident. There are receptors for the constant region of antigen-specific factors on the accessory cell surface, and the antigen cross-links the receptors via these antigen-specific factors.



thresholds in the amount of specific T cell factors that can result in switching from the virgin to the immune state. In the unperturbed system there is a mixture of T cell factors of many different specificities on the A cell surface, consistent with the A cell being non-specific.

If a very small amount of antigen is used, it would suffice to stimulate some high affinity positive clones to proliferate and secrete specific factors, but may not suffice to cause immediate activation of the A cell, since the A cell would initially remain relatively non-specific. Proliferation of a small number of high affinity antigen-specific clones would however eventually lead to the arming of A cells preferentially with high affinity specific T cell factors, and lead to the stimulation and proliferation also of the corresponding antiidiotypic clones. Mutual stimulation of the antigen-specific and antiidiotypic clones, with the A cell surface as a catalyst, leads to the suppressed state for the antigen. The activation of the A cells would suffice to cause the secretion of the non-specific factor involved in T cell proliferation (IL-1), but would not suffice to cause the secretion of the non-specific factor that is involved in B cells differentiating to become plasma cells. When the A cell becomes armed with antigen-specific factor, it provides an immunogenic array of factors that stimulate antiidiotypic cells, providing the receptor for the factor on the A cell is at least divalent. Similarly, antiidiotypic factors on the A cell would stimulate idiotypic T cell clones. The A cell catalyzes co-selection of antigen-specific and antiidiotypic T

cells. Hence the model explains the phenomenon of the induction of unresponsiveness with low doses of antigen, which is the simplest form of switching between stable states; see Figure 10-13.

The induction of an immune response according to the model involves the activation of the A cell and the production of the non-specific differentiation factor as illustrated in Figure 10-13. A larger dose of the antigen than that used for low dose tolerance results in more rapid and effective arming of the A cell (and/or the antigen) with antigen-specific T cell factors, and thus leads to greater A cell activation by the antigen via the factors. While in the case of low dose tolerance the accessory cells make primarily IL-1, this time they are activated also to make the factor needed for the B cells to differentiate to become antibody-secreting plasma cells. Only those B cells that have been activated by the cross-linking of their receptors express the receptor for the non-specific factor. The antigen-specific antibodies secreted by B cells both eliminate the antigen and kill antiidiotypic cells, thus leading the system to the immune steady state with an elevated levels of antigen-specific cells and a depressed level of antiidiotypic cells.

Responses to hapten-carrier conjugates

Figure 10-14 shows interactions for an immune response to a hapten-carrier conjugate, for the case of the carrier being a small protein. Carrier-specific T cell factors are bound via their C regions to the receptor for specific factors on the A cell, while the V region of the factor is bound to the carrier part of the antigen. The hapten interacts with the specific receptor of the B cell. Cross-linking of the specific receptor of the B cell causes the B cell to proliferate and express the receptor for the non-specific differentiation factor.

A very high dose of antigen rapidly stimulates too many T cells, such that both the antigen and the A cell would become "armed" with antigen-specific T cell factor. The factors then cannot however form a bridge between antigen and A cell, because the antigen is blocked by the excess specific factor, as shown for a response to a hapten-carrier conjugate in Figure 10-15. The activation of the A cell is thus inhibited relative to the level of activation in the immune response, and the B cells again do not receive the required "second signal". The armed A cells nevertheless induce low affinity antiidiotypic T cells (that are not so effectively blocked by antigen-specific factors) to proliferate, leading again to the suppressed state via co-selection of antigen-specific and antiidiotypic T cells. There is again sufficient activation of the A cell to cause the production of IL-1, giving T cells a second signal for proliferation.

Figure 10-13. Model for the induction of low dose tolerance. The antigen stimulates antigen-specific T cells to secrete antigen-specific factors, that bind to the surface of non-specific accessory cells. On the A cell surface they provide an immunogenic array for antiidiotypic T cells, providing the receptor for the factor is at least divalent, as shown in Figure 10-12. Stimulation of the antiidiotypic T cells results in the A cell becoming armed with both antigen-specific and antiidiotypic factors, so that the A cell is a catalyst for the mutual stimulation of antigen-specific and antiidiotypic T cells. This catalyst role is enhanced by the fact that the A cell is activated to produce IL-1, a lymphokines that gives the T cells a second signal for proliferation. Proliferation of both antigen-specific and antiidiotypic T cells puts the system on a trajectory towards the suppressed state (Figure 10-2).

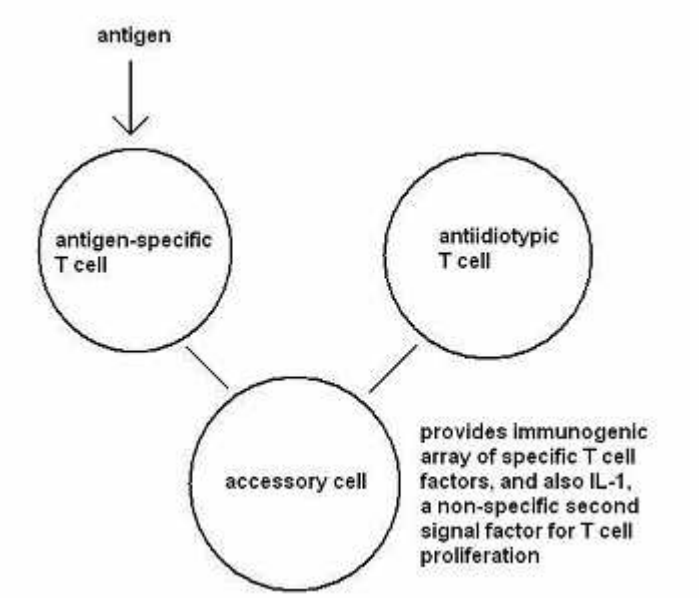


Figure 10-14. Model for the immune response to a hapten-carrier conjugate, for the case that the carrier is a small protein. Antigen-specific T cell factors are bound to a receptor for the factors on a non-specific accessory cell. The "C" denotes the constant part of the carrier-specific factors; the other end is the V region that binds to the carrier. The B cell has receptors that bind to the hapten, and the hapten-carrier is a bridge between the B cell and the accessory cell. The stimulation of the B cell by the cross-linking of its receptors results in the expression of a receptor for the non-specific factor. The non-specific factor gives the B cell a second signal, that induces it to differentiate to become an antibody producing plasma cell. Reproduced from G. W. Hoffmann (1978) in "Theoretical Immunology", G.I. Bell, A.S. Perelson and G.H. Pimbley (eds.) Marcel Dekker, N.Y., 571-602.

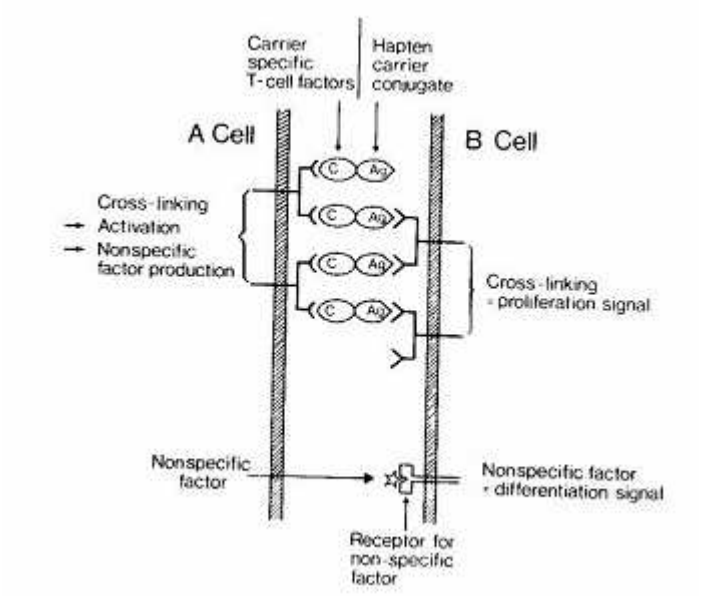
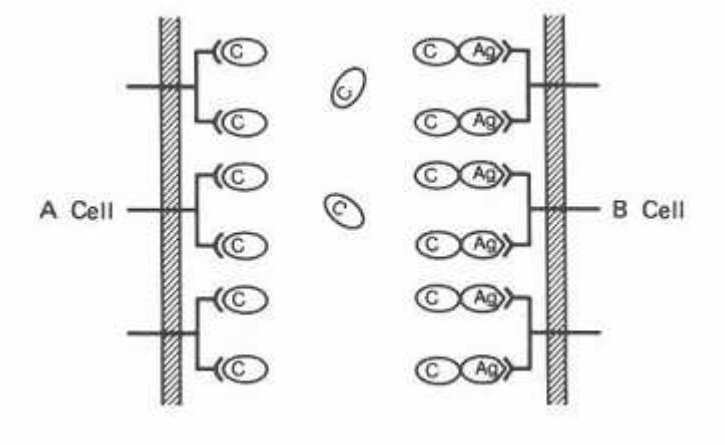


Figure 10-15. High dose tolerance for the case of a hapten-carrier conjugate. Excess carrier-specific T cell factor inhibits the activation of the accessory cell by the antigen via specific factors. Specific T cell factors are shown binding via their constant (C) regions to a divalent receptors on the A cell, providing an immunogenic array (capable of cross-linking) for antiidiotypic T cells. The hapten of the antigen binds to a B cell receptor, while a carrier determinant binds to the variable part of the specific T cell factor. The accessory cell is again a catalyst for the mutual stimulation of carrier-specific and antiidiotypic T cells, with IL-1 providing a non-specific signal for proliferation of the T cells. Reproduced from G. W. Hoffmann (1978) in "Theoretical Immunology", G.I. Bell, A.S. Perelson and G.H. Pimbley (eds.) Marcel Dekker, N.Y., 571-602.



A prediction of this model is that there is a receptor on A cells for the constant part of antigen-specific T cell factor. This receptor is multivalent, most simply divalent, in order that the A cell can exert its catalyst role in the co-selection of antigen-specific and antiidiotypic T cells, leading to the suppressed state.[128]

In conclusion, the model leads directly to explanations for low dose tolerance, an immune response and high dose tolerance. At low and high doses of antigen there is relatively little activation of the A cell. The A cell surface can then nevertheless constitute a catalytic surface for the mutual stimulation of antigen-specific and antiidiotypic T cells by adsorbed specific T cell factor, and thus the induction of the suppressed state. The mechanism is similar to the way that activation of mast cells and basophils via IgE occurs at intermediate levels of a divalent hapten, but not at much lower or higher levels of the divalent hapten.[122,123] In that system activation can also be inhibited by monovalent hapten. This model for low and high tolerance is however more complex than the IgE system, in that it includes the A cell catalyzed co-selection stimulation of antigen-specific and antiidiotypic T cell clones in both low dose and high dose tolerance.

Antigenic competition

The symmetrical network theory provides an explanation for the phenomenon of antigenic competition, which was also mentioned in chapter 6. The immune response to an antigen (say "X") inhibits the response to a second antigen (say "Y") given shortly after the application of X. This can be understood in the context of the symmetrical network theory in terms of what happens at the A cell surface. The A cell surface is a potent self antigen, especially for helper T cells, since it expresses MHC class II molecules, and helper T cells are biased towards having affinity for MHC class II (see chapter 12), and also because it provides T cells with a second signal for proliferation. The specificity of the A cell is however a dynamic variable, since it has the specificity of the sum of all the adsorbed specific T cell factors on its surface. The introduction of the foreign antigen X stimulates X-specific T cells and thus changes the spectrum of specific factors on the A cell surface. By the law of mass action, an increase in the concentration of X-specific T cell factors causes the A cell to tend to adopt an anti-X specificity.

We can invoke the concept of shape averaging to define the specificity of a diverse population. The A cell stimulates especially those T cells to proliferate that have specific receptors complementary to the average shape of the specific factors that are on the A cell surface, and that are not blocked by complementary T cell factors. The average shape of the antigen-specific T cell

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[128] Prediction

factors is complementary to the antigen and the average shape of the antiidiotypic T cell factors resembles the antigen. The relevance for antigenic competition is then as follows.

The antigen X causes the stimulation of a set of antigen-specific T cells. The specific factors made by these bind to the A cell surface. The array of antigen-specific factors (anti-X) causes the stimulation of antiidiotypic (anti-anti-X) cells. We then have two mutually specific sets of cells stimulating each other, and also stimulating the A cell to make IL-1. If there is enough stimulation of the A cell, it also produces B cell differentiation factor, and there is an anti-X immune response. If the A cell is not activated, it can still act as a catalyst for the mutual stimulation of anti-X and anti-anti-X cells, taking the system to the suppressed state for the antigen X. The positive/negative stimulation of the T cells defines an axis called, say, "anti-X/anti-anti-X" in shape space. If we now add the second antigen Y to the system, it has its own axis "anti-Y/anti-anti-Y" in shape space, and the activation of that axis has to compete with activation along the axis defined by X. The specific factors of the first antigen are however already established on the surface of the A cell, and the factors of the second antigen cannot effectively compete with them, because of the autocatalytic nature of the positive/negative (anti-X/anti-anti-X) stimulation. We therefore do not get activation of the system along the anti-Y/anti-anti-Y axis, (including activation of B cells with anti-Y specificity) and the response to Y is weak at best.

IgM enhancement and IgG inhibition of immune responses

Antigen-specific IgM antibodies injected together with antigen result in the enhancement of the immune response, while antigen-specific IgG antibodies do the opposite.[129] The effect of IgM is readily explained; these antibodies, together with complement, efficiently kill antiidiotypic clones, allowing antigen-specific clones to proliferate and differentiate to secrete antibodies.

The inhibitory role of antigen-specific IgG antibodies is not so readily accounted for, especially in view of the fact that, in our model, antigen-specific IgG also kills antiidiotypic clones in the immune state. On the other hand, the bell-shaped dose dependence for bivalent hapten triggering mast cells and basophils to release histamine emphasizes the non-linear system-response behaviour of a system that involves cross-linking of receptors. It is plausible that the IgG is able to cross-link receptors of many antiidiotypic T cells for which they have low affinity, such that the cross-linked receptors stimulate the cells, without pairs of adjacent IgG antibodies being present on the cell

[129] C. Henry and N. K. Jerne (1968) Competition of 19S and 7S antigen receptors in the regulation of the primary immune response. J. Exp. Med. 128, 133-152. “19S” and “7S” refer to IgM and IgG antibodies respectively.

surfaces, that could bind complement and lead to the killing of the cells. The antigen stimulates antigen-specific T cells, while the IgG antibodies stimulate antiidiotypic T cells. This combination could trigger co-selection of the two populations and thus nudge the system towards the suppressed state rather than the immune state. That is, antiidiotypic T cells stimulate the antigen-specific T cells and vice versa (via specific factors on the A cell surface), moving the system toward the suppressed state.

Both activating and tolerizing signals?

In immune network theory we have a type of interaction that is missing in non-network models, namely (of course) V-V interactions. On the other hand, we are able to dispense with a type of interaction that is common in non-network theorizing, namely "tolerizing signals." Many non-network papers have been published suggesting, for example, that an antigen is able to interact with a cell in a way that actively switches it off rather than on. In our model we have only stimulation by antigen, together with stimulation, inhibition (blocking) and killing (elimination) by antibodies, specific factors or cells, via V regions, without any provision for cells being switched off directly by antigen. Tolerance is related to population levels, rather than being a property of the system at the level of individual cells interacting with antigen.

Kinetics of induction of tolerance in T cells and B cells

We will now consider an experiment that has traditionally been considered to demonstrate tolerance at the level of individual cells, rather than at the level of populations of cells. Chiller, Habicht and Weigle[130] reported in a famous paper that unresponsiveness can be induced in both B cells and T cells. They worked with a system for which they had both a tolerogenic and an immunogenic form of the antigen, namely human gamma globulin (HGG; "gamma globulin" is an old term for IgG). The tolerogen was deaggregated human gamma globulin (DHGG), and the immunogen was aggregated human gamma globulin (AHGG). The tolerogen is produced by ultracentrifugation of HGG, which causes any aggregates to sink to the bottom of the tube. Mice can be made unresponsive ("tolerized") by immunizing them with the tolerogen. "Unresponsive" means that the mice do not make anti-HGG antibodies in response to an injection of the immunogen. Chiller et al. investigated the question how rapidly the T cells and B cells become unresponsive following an injections of the tolerogen, and how long the unresponsive state persists.

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130 J. M. Chiller, G. S. Habicht and W. O. Weigle (1971) Kinetic differences in unresponsiveness of thymus and bone marrow cells. Science 171, 813-815.

In order to investigate the cellular basis of tolerance in this system, mice were irradiated (to eliminate their own lymphoid cells) and repopulated with either T cells from tolerogen treated mice and B cells from normal mice, or T cells from normal mice and B cells from tolerogen treated mice (Figure 10-17a) Controls with normal T cells and normal B cells responded to the immunogen, and all groups of mice responded to the control antigen turkey gamma globulin. The kinetics for the induction of unresponsiveness differed in T cells and B cells, with unresponsiveness being induced rapidly (within a day) in T cells and within 21 days in B cells (Figure 10-17b). The completely unresponsive state of the T cells persisted for the seven week duration of the experiment, while the B cells fully regained their responsiveness to the immunogen by the end of that period. These results were widely interpreted in terms of there being two kinds of T cells and two kinds of B cells specific for HGG, namely "responsive" and "unresponsive", with the deaggregated antigen can somehow causing the individual cells to switch from being responsive to being unresponsive. This experiment is featured in many textbooks of immunology.

Our model does not include such intrinsically responsive and unresponsive cells, but it does include both antigen-specific and antiidiotypic cells. Can the model account for the data of Chiller et al., including the differences between T cells and B cells? The perturbation of the T cell side of the system is presumably rapid because the antigen stimulates the rapid release of positive specific T cell factors, that bind to the A cell surface, and these provide antiidiotypic T cells with a stimulatory array. The ensuing release of antiidiotypic specific T cell factors leads to a mixture of positive and negative specific factors on the A cell surface, that is stimulatory for both antigen-specific and antiidiotypic T cells. This combination would precisely define an axis (or direction) for the system, and the system would then autonomously move towards the suppressed state for HGG. We have a two-member *positive feedback loop*, since antiidiotypic cells are selected by antigen-specific factors secreted by antigen-specific cells, and antigen-specific cells are selected by antiidiotypic factors made by antiidiotypic cells (in addition to antigen). These two populations of T cells define an axis in "shape space", in contrast to a single population, which would define only a point in shape space.[131] An axis is more specific than a point because two points are needed to define an axis. There is co-selection of antigen-specific T cells and antiidiotypic T cells, that is catalyzed by the A cell, with the A cell providing the T cells with the non-specific factor for proliferation. The T cell part of the system does not have to be in the suppressed state at the time when the cells are transferred. It only has

[131] Two similar shapes map close to each other in shape space, while two very different shapes map to points that are correspondingly distant from each other. In chapter 19 an experimental method for mapping samples containing immune system V regions in a highly dimensioned shape space is described.

to be out of the starting blocks and moving in a precisely defined direction towards the suppressed state, as defined by the combination of antigen-specific and antiidiotypic specificities. It must be out of the zone of attraction of the virgin state, which may not be very large. The rapid change to being on a trajectory to the suppressed state indicates that the secretion of specific T cell factors occurs very quickly. The receptors for antigen-specific T cell factors on the A cell and the catalytic role of the A cell, also due to its secretion of IL-1, makes the co-selection process for antigen-specific and antiidiotypic T cells more efficient than co-selection of antigen-specific and antiidiotypic B cells. Consequently, the unresponsive state is very rapidly induced in T cells, and is more stable.

T cells are tolerized at lower doses of antigen than B cells

Our model includes the idea that the threshold for activation of the A cell to produce the non-specific proliferation factor (IL-1) for antigen-specific and antiidiotypic T cells is lower than the threshold for producing the non-specific differentiation factor for B cells. This postulate would account for the fact that specific tolerance of T cells can be generated with lower levels of antigen (by a factor of 100 to 1000) than is needed for inducing tolerance in B cells.[132] At low doses there can then be enough stimulation of the A cell to facilitate the proliferation of antigen-specific and antiidiotypic T cells, but not enough activation of the A cells for them to produce the B cell differentiation factor. There is then effective co-selection of antigen-specific and antiidiotypic T cells, without the production of antibodies, setting the system on a trajectory towards the suppressed state.

Low doses of antigen can induce suppressor T cells

Support for the role of T cells in low dose tolerance comes from the work of Kölsch and his collaborators.[133] Working with the phage fd as an antigen in the mouse, they found that multiple low doses of fd (5 million phage particles, about one picogram, per dose) induce tolerance. The tolerance could not be overcome by injection of normal spleen cells, consistent with an active suppression mechanism. The suppressed state was also observed in irradiated recipient mice that received cells from low dose treated mice, fetal liver cells and normal thymocytes. It was shown that the suppression is due to T cells.

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132 J. G. Howard and N. A. Mitchison (1975) Immunological tolerance. Prog. Allergy 18, 43-96.

133 E. Kölsch, R. Stumpf and Weber, G. (1975) Low zone tolerance and suppressor T cells. Transpl. Rev. 26, 56-86.

These are important results because they link the phenomena of low dose tolerance and suppressor T cells.

On low doses, suppressor T cells and criss-cross specificity

To my knowledge, the phenomenon of low dose tolerance (including ultra-low dose tolerance) has not been demonstrated in experiments with criss-cross specificity controls. The same is true for experiments that demonstrate the link between lose dose tolerance and antigen-specific suppressor T cells. Each investigator has typically worked with just one antigen. Even thirty years later, it would be worthwhile to demonstrate criss-cross specificity in such experiments, since that would increase by a notch the level of rigour at which these phenomena have been established. These very simple phenomena are important pieces in the overall picture of experiments supporting immune network theory, so confirmation at the highest level of rigour in at least one experimental system would be helpful.

A low dose of antiidiotype can induce specific suppressor T cells

The co-selection of idiotypes and antiidiotypes can account for the induction of the suppressed state observed in the experiments of Eichmann shown in Figure 6-4. The transfer of small numbers of T_+ and T_- cells from a suppressed animal would be able to autocatalytically induce the suppressed state in the recipient, with T_+ factors stimulating host T_- cells and vice versa.

Induction of the suppressed state using an idiotype?

A prediction of the theory is that it should be possible to induce the suppressed state also using the idiotype, since the suppressed state is symmetrical with respect to the idiotype and the antiidiotype.[134] It would have to be a low dose of the idiotype (e.g. IgG idiotype) to ensure that it acts in a stimulatory fashion (cross-linking receptors on antiidiotypic T cells, and causing the secretion of antiidiotypic T cell factors), rather than killing the antiidiotypic T cells. The latter would occur if there is enough idiotype to enable pairs of IgG molecules to bind next to each other on T cell surfaces and trigger killing with complement.

Antigen-specific factors can have either helper or suppressive effects

In our model we have not distinguished between antigen-specific factors that help immune responses and factors that specifically suppress immune

[134] Prediction.

responses. Experimentally one typically sees either help or suppression. This is not surprising, since a factor preparation typically contains a spectrum of specificities, that may be tuned by the method of preparation to preferentially help or suppress. The effect seen may also depend on the assay used to detect the factors. Assays that are designed to detect T cell help may not be capable of demonstrating suppressive effects and vice versa. The method of preparation, the antigen, genetics (the animal strains used) and the assay method are among the parameters determining whether help, suppression, or no effect is seen experimentally.

The reported physical properties of helper and suppressor factors are very similar. They have similar molecular weights (about 50,000), each factor must have a V region, and must presumably have a C region, since in several cases they have been shown to bind to A cells, and the A cell must therefore have a receptor for specific factors. Both antigen-specific helper factors and antigen-specific suppressor factors have been found to bind to A cells. Among the first reported antigen-specific factors was a factor called specific macrophage arming factor, that binds to the surface of macrophages[135].

Extensive experimentation confirms the existence and importance of specific suppressor factors

A large number of experiments by many immunologists have confirmed the existence and potent regulatory effects of antigen-specific T cell factors.[136] This is an important point, because the investigation of these molecules has gone out of fashion, and many younger immunologists are unaware of their existence! They are out of fashion largely, I believe, because of the impact that the I-J paradox had on cellular immunology. The I-J phenomenon, the I-J paradox, and how the I-J paradox can be resolved in the context of the symmetrical network theory, are discussed in 13.

Antigen-specific T cells, antiidiotypic T cells, antigen-specific T cell factors and antiidiotypic T cell factors play roles in suppression

Detailed analysis of suppression has shown that there are at least two populations of T cells involved, namely antigen-specific, called "Ts1" and antiidiotypic T cells called "Ts2", together with their corresponding antigen-

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135 R. Evans, C. K. Grant, H. Cox, K. Steel and P. Alexander (1972) Thymus-derived lymphocytes produce an immunologically specific macrophage-arming factor. J. Exp. Med., 136, 1318-1322.

136 Reviewed in T. Tada (1984) Help, suppression and specific factors. In Fundamental Immunology, W. E. Paul Ed. (1st edition) Raven Press, New York, 481-517.

specific and antiidiotypic factors, called TsF1 and TsF2,[137,138] strongly supporting the symmetrical network theory. It is noteworthy that reports of antiidiotypic T cells and antiidiotypic specific factors came after the publication of the theory, providing crucial confirmation of a central aspect of the theory.[139] There is furthermore evidence of a role for anti-antiidiotypic suppressor T cells ("Ts3"), that I will describe and incorporate into the theory in chapter 17.

Allotype suppression

Serological markers are epitopes detectable by antibodies. Serological markers present on antibodies include idiotypes and allotypes. Idiotypes are markers on the V regions, while allotypes are markers present on all antibodies of a given inbred strain, and are typically present on the C regions of antibodies. They can also be on a relatively constant part of the V domain. Different strains of mice can have different allotypes. For example, the mouse strain Balb/c has the IgG allotype a ("Ig-1^a"), while the strain SJL has the IgG allotype b. We saw in chapter 6 that mice can be manipulated, such that clones producing antibodies with a given idiotype are specifically suppressed, and that the same is true for clones producing a given allotype.

As described in chapter 6, allotype suppression has been extensively studied in (Balb/c x SJL) F1 mice. If a female Balb/c mouse (Ig-1^a) is immunized with IgG antibodies from an SJL mouse, and makes anti-(Ig-1^b allotype) antibodies, and is then mated with an SJL male, many of the offspring make antibodies that are exclusively of the a allotype.**Error! Bookmark not defined.** Some animals transiently produce some b allotype antibodies, and then become chronically suppressed. The experiment of Figure 6-5 is strong evidence for suppressor T cells being responsible for the absence of b allotype antibodies in the allotype-suppressed off-spring.

The suppression of half the IgG antibodies in an animal is a dramatic effect that can be understood in the context of the symmetrical network theory. It is presumably important that IgG antibodies from the anti-(allotype b) immune mother circulate in the newborn, and that both a small amount of allotype b

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137 M.-S. Sy, M. H. Dietz, R. N. Germain, B. Benacerraf and M. I. Greene (1979) Antigen- and receptor-driven regulatory mechanisms. IV. Idiotype bearing I-J^+ suppressor T cell factors (TsF) induce second order suppressor T cells (Ts2) which express antiidiotypic receptors. J. Exp. Med. 151, 1183.

138 Y. Hirai and A. Nisonoff (1980) Selective suppression of the major idiotypic component of an anti-hapten response by soluble T cell-derived factors with idiotypic or antiidiotypic receptors. J. Exp. Med. 151, 1213-1231.

139 Confirmed prediction.

(possibly solely as receptors) and anti-(allotype b) antibodies are present. This combination stimulates the establishment of the suppressed state, with co-selection of elevated levels of allotype b specific and allotype b mimicking T cells, providing a high level of T cell connectivity specific for the allotype, that inhibits the secretion the allotype b antibodies. In a normal animal, on the other hand, a high level of allotype b antibodies, acting alone, would kill the anti-(allotype b) T cells, and the suppressed state could not be established.

The relationship between immunogenicity and tolerogenicity

Flagellin is both a potent antigen and a potent tolerogen, which shows that a substance can be both tolerogenic and immunogenic. This fits well with our network model, since the ability to cross-link receptors is essential in both cases, and is likely to be the common factor. This raises the question of how a small, monomeric protein can be immunogenic. The ability to cross-link receptors may occur when the a monomeric antigen is effectively polymerized in vivo, by binding to complementary cell surface receptors, for example the specific receptors of antigen-specific B cells. More specifically, a monomeric protein with two antigenic determinants "X" and "Y" can be converted into a polymeric form capable of cross-linking the receptors of X-specific lymphocytes by first binding to divalent, antigen-specific receptors of Y-specific B cells. Then the monomeric protein becomes an array of a divalent form of the monomers.

Tolerance without suppression

It is possible to induce specific tolerance to an antigen without inducing suppressor T cells.[140] This result may at first glance seem to disprove our model, since the only unresponsive state we have mentioned is the suppressed state with elevated levels of antigen-specific and antiidiotypic cells. In order to address this apparent discrepancy, we need to consider how many of the experiments on antigen-specific tolerance are done.

An assumption underlay the interpretation Chiller et al. gave to their experiment (see above "Induction of tolerance in T cells and B cells"), namely that the cells are either "tolerant" or not at the time of the transfer into an irradiated recipient. Would a cell that is tolerant be distinguishable at the microscopic level from one that is not tolerant? Or do we rather have tolerance at the population level? The population dynamics or network view is that there has been a change in populations as a result of the antigen priming, such that at some time point after challenge in the new host there is or is not a response.

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140 J. M. Chiller, G. S. Habicht and W. O. Weigle (1970) Cellular sites of immunological unresponsiveness. Proc. Nat. Acad. Sci. USA 65, 551-556.

What we have at the time of the transfer is a difference in the initial conditions for the dynamics within the adoptive host (that is, differences in the numbers of both idiotypic and antiidiotypic cells of various cell types). Our read-out with the assay comes several days later, after the dynamics have further changed the system.

Table 10-2 illustrates the complexity of the situation, and the dynamic nature of the levels of the antigen-specific and antiidiotypic populations, such that a trajectory towards the suppressed state may or may not be established at the time of the transfer of cells. There is also a lot more going on than just changes in actual cell population levels. Immediately following immunization there may not yet be any changes in cell population levels, but the levels of antigen-specific and antiidiotypic T cell factors may change very quickly, contributing to determining the dynamical path that is followed.

To conclude that the T cells or the B cells are either "tolerant" or "not tolerant" is therefore an unrealistic simplification. The fact that it takes 6 weeks to establish complete suppression in the idiotype suppression system of Eichmann (Figure 6-4) is also consistent with the concept that the trajectory is important, and the trajectory is certainly a function of both idiotypic (antigen-specific) and antiidiotypic cells, not only of the antigen-specific cells.

Responses to T-independent antigens

Some antigens do not need a discernible amount of T cells in order to induce an immune response. Such antigens are typically polymeric, flexible molecules that are presumably very efficient at cross-linking receptors, even when the affinity of one unit of the polymer for the specific B cell receptor is very low. They may thus cross-link the receptors for specific factors on the A cell, without any need for specific T cell factors to act as a bridge. Alternatively, a small amount of specific T cell factors may be present on the A cell surface, and the very flexible T-independent antigen may cooperatively bind to these factors and crosslink the receptors, stimulating the A cell to secrete non-specific differentiation factor for B cells.

Maintenance of tolerance in the absence of the antigen

The suppressed state for an idiotype in the case of the Eichmann system (Figures 6-4a and 6-4b) did not need any antigen to be stable. In the system of Chiller et al. (Figure 10-17a and Figure 10-17b), T cell tolerance in the irradiated recipients and tolerance in the tolerogen treated mice is stable without a maintenance regimen of antigen. These results make sense in terms of mutually stabilizing stimulatory interactions between antigen-specific and antiidiotypic

Table 10-2. Dynamics of populations 1 and 2 cells (x_1 and x_2, antigen-specific and antiidiotypic respectively) following administration of an antigen in tolerogenic form. This table is also a gross simplification, since a more complete description would include enumerating antigen-specific and antiidiotypic cells of multiple types, as well as their soluble products, namely antibodies and specific T cell factors.

	Population 1 cells (antigen-specific)	Population 2 cells (antiidiotypic)
Pre-immunization	x_{11}	x_{21}
Immediately after immunization	x_{12}	x_{22}
24 hours after immunization	x_{13}	x_{23}
At time of transfer	x_{14}	x_{24}
Minimal levels needed at the time of transfer for unresponsiveness to eventually be seen in the assay	x_{15}	x_{25}
At time of assay	x_{16}	x_{26}

T cells, making the presence of the antigen unnecessary. The findings are incompatible with theories of tolerance that involve antigen being needed to directly switch clones off rather than on.

Breaking tolerance with a cross-reactive antigen

Weigle found that tolerance can be broken using a cross-reactive antigen, that is, an antigen similar to but not identical to the antigen used to induce specific tolerance.[141] This can be understood by considering how the specificity of a suppressed state may compare with the specificity of immunity. The tolerogen induces elevated levels of idiotypes and antiidiotypes specific for that antigen, especially T cells. These define an axis in our shape space. Let us call the tolerogen X and the cross-reactive antigen W. If we are able to obtain an immune response to W, this shows that the suppressed region of shape space is relatively limited, otherwise the response to W would also be suppressed. The suppressed state is defined by both anti-X and anti-anti-X, which together define an axis in shape space. Induction of the suppressed state involves co-selection of two populations, in contrast to the induction of

141 W. O. Weigle (1973) Immunological unresponsiveness. Adv. Immunol. 16, 61-122.

immunity, that involves the positive selection of one population and the elimination of another. The anti-W immune response is thus defined by a point in shape space, and is less specific than the anti-X/anti-anti-X axis of the tolerant state for X. The broader specificity of the anti-W response means anti-W can eliminate not only anti-anti-W but also anti-anti-X, leaving anti-X free to respond. Hence the response to X can break tolerance to W.

The above idea may however be less than the full story, since there is evidence that only a small fraction of antigen-specific B cells normally respond to antigen by making antibodies. Some assays reveal only one in 100,000 B cells responding to a given antigen, while when spleen cells are stimulated by mitogens (that stimulate B cells non-specifically), 1 in 1000 make antibodies specific for the same antigen. Thus most B cells are somehow suppressed, and, as Jerne put it, the immune response appears to be an "escape from suppression."

Auto-antiidiotypes, and why we don't always detect them

The universe of antibodies and the universe of antigens are not two separate universes. Rodkey[142] showed that an animal has the capacity to make antibodies specific for the idiotypes of its own antibodies. He immunized rabbits with a hapten coupled to a carrier and purified the anti-hapten antibodies. From the antibodies from each rabbit he prepared $F(ab')_2$ fragments, polymerized these to make them more immunogenic, and injected each rabbit with its own specific $F(ab')_2$ fragments together with an adjuvant. Each of the rabbits made antiidiotypic antibodies specific for its own anti-hapten antibodies. This experiment showed that the rabbit had the genetic capacity to make antiidiotypes to its own idiotypes, and was regarded as important in lending credence to the network hypothesis.

However, the experiment left many immunologists skeptical. The experiment was artificial, since it involved using artificially polymerized preparations of the antibodies. Furthermore, one sometimes heard the statement "I don't see antiidiotypes in my system", and immunologists are naturally most convinced by what they see experimentally themselves. In the context of the symmetrical network theory, however, one would *not expect* to see antiidiotypes at an elevated level in most immune responses. This is because the immune state of the theory is one in which the idiotypes have eliminated the antiidiotypes. On the contrary, the theory predicts that the immune response should cause a decrease in the level of antiidiotypes relative to the level

142 L. S. Rodkey (1974) Studies of idiotypic antibodies. Production and characterization of auto-antiidiotypic antisera. J. Exp. Med. 139, 712-720.

observed in the virgin state.[143] The fact that Rodkey was able to nevertheless produce auto-antiidiotypes shows that the elimination of antiidiotypes is not complete (which is also the case in the theory). With time the level of antibodies to the antigen wanes, and there is a corresponding partial resurgence in the level of antiidiotypes. Challenge with the idiotype together with an adjuvant can then evoke an antiidiotypic response.

A subsequent paper by Brown and Rodkey[144] showed that antiidiotypes can be made following repeated immunization with an antigen, and supported these ideas. A rabbit was injected three times with *Micrococcus lysodeikticus* vaccine over a 31 month period. The first injection caused a strong anti-micrococcus response together with a strong anti-IgG (rheumatoid factor) response. The second immunization included anti-micrococcus, antiidiotypic antibodies specific for idiotypes present on anti-micrococcus antibodies made in the first response, and Fc-specific anti-IgG rheumatoid factor. The regulatory effect of the antiidiotypes was apparent from the fact that anti-micrococcus idiotypes present in the response to the first immunization were reduced in quantity or absent when the corresponding antiidiotypes were present. The third round sera contained no detectable antiidiotypic antibodies, but did contain anti-micrococcus antibodies with idiotypes that were present in the first and second round immunizations.

This paper provides compelling support for the relevance of idiotypic network regulation in ordinary immune responses, and can be adequately interpreted in detail in the context of the postulates of the symmetrical network theory as follows. The initial production of anti-micrococcus antibodies results in the arming of antiidiotypic cells with these antibodies. This provides an immunogenic array of the constant part of IgG that triggers a rheumatoid factor response, as observed in the first round of immunization. Rheumatoid factor may then play a role in the formation of multivalent complexes of anti-micrococcus antibodies, that stimulate the production of antiidiotypic antibodies, and these are detected following the second round of immunization. The antiidiotypic antibodies so produced can kill cells expressing the corresponding idiotypes, preferentially suppressing the expression of those particular idiotypes. A further immunization with the antigen boosts the production of anti-micrococcus antibodies, and clones are selected that preferentially recognize both the antigen and the antiidiotypes that are present. The antibodies made by these clones then essentially eliminate the corresponding antiidiotypic clones, yielding the immune state of the theory.

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[143] Prediction.

[144] J. C. Brown and L. S. Rodkey (1979) Autoregulation of an antibody response via network-induced auto-anti-idiotype. J. Exp. Med. 150, 67-85.

The idiotypes that were present in the first and second round have then "conquered" the corresponding antiidiotypes.

Dominant idiotypes

In many of the systems of idiotypic regulation that have been investigated, a particular serologically defined idiotype is reproducibly produced in response to a given antigen in only one or a few mouse strains. There is typically a linkage between the expressed idiotype and B cell V region genes, especially genes for heavy chain V regions. Such reproducibly produced idiotypes are called dominant idiotypes. There is evidence that dominant idiotypes emerge during the development of the idiotypic network during ontogeny.[145] The set of B cell V genes thus significantly influences the repertoire of expressed idiotypes.

Auto-antiidiotypes in some immune responses to protein antigens.

Evidence of specific auto-antiidiotypes ("AAI") in immune responses to three protein antigens, namely bovine serum albumin (BSA), was obtained by Robert Forsyth in my laboratory.[146] Chickens were immunized several times with one of three protein antigens, namely bovine serum albumin (BSA), keyhole limpet hemocyanin (KLH) and diphtheria toxoid (DT). Antibodies present in the immune sera following the final immunizations were purified using columns of antigen coupled to Sepharose beads. Specific antibody bound to the antigen in the columns, and was then eluted from the column using dilute acid. Forsyth found that column purified, biotinylated anti-BSA bound to column purified anti-BSA in an ELISA much more than did similarly purified, biotinylated anti-DT or biotinylated KLH. Similarly, biotinylated anti-DT bound to anti-DT more than biotinylated anti-BSA or biotinylated anti-KLH did. Finally, biotinylated anti-KLH bound more to anti-KLH than biotinylated anti-BSA or biotinylated anti-DT did (three-way criss-cross specificity). The amount of AAI activity in the affinity purified chicken anti-BSA antibodies was similar to the amount of anti-BSA activity.

The AAI response was strongest for BSA, so Forsyth investigated the phenomenon further with mice immunized with BSA. The mice made anti-BSA antibodies that reacted specifically with affinity purified chicken anti-BSA but not chicken anti-KLH antibodies. Hence these sera contained AAI

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[145] J. F. Kearney, M. Vakil and N. Nicholson (1987) Non-random VH gene expression and idiotype-antiidiotype expression on early B cells. In: Evolution and Vertebrate Immunity: The Antigen Receptor and MHC Gene Families. G. Kelsoe and G. Schulze Eds. Texas University Press, Austin, 175-190.

[146] R. B. Forsyth and G. W. Hoffmann (1990) A study of auto-antiidiotypes to BSA. J. Immunol., 145, 215-223.

antibodies with specificity that crosses species. Since chickens and mice are distantly related phylogenetically, the fact that mouse antiidiotypes react with chicken anti-BSA seems unlikely to be due to similarity in V region genes between mice and chickens. In other words, this is unlikely to be a case of germ line V region determined clonal dominance. A more plausible explanation is that the antiidiotypes produced in this system resemble the antigen in both mice and chickens.

The activity seen in these experiments could in principle be due to column purification of complexes of antigen-specific and antiidiotypic antibodies. However, antiidiotypic antibodies, that are present in the same amount as antigen-specific antibodies, might be expected to inhibit the binding of the antigen-specific antibodies to the column. This inhibition would involve two V-V interactions between the two antibodies, which together would be stronger than a complex involving an anti-BSA IgG binding to the column with one arm, and binding to an antiidiotypic antibody with the other arm.

An intriguing possibility that would automatically account for this approximate equality between antigen-specific and antiidiotypic antibodies, is that the immunizations led to the production of antibodies that are both antigen-specific and antiidiotypic. The chickens that were immunized with BSA received BSA in adjuvant (complete Freund's) in week 1, then BSA in buffered saline in week 2, and BSA in incomplete Freund's adjuvant in week 3. At week 8 they were immunized again with BSA in complete Freund's adjuvant. The AAI were produced following the final immunization. By the time of that immunization the chicken had made a significant IgG anti-BSA response. In the context of our theory, these anti-BSA antibodies would kill antiidiotypic T cells for which they have high affinity, but they would stimulate antiidiotypic T cell clones with lower affinity, and we can expect the A cells to be significantly armed with such antiidiotypic T cell factors. (The affinity would have to be such that the IgG cross-links antiidiotypic T cell receptors, causing release of specific factor, while there is not a significant number of pairs of IgG antibodies that would bind complement and lead to killing of the T cell.) The perturbation of the system with BSA then stimulates the renewed production also of antigen-specific T cell factors. The presence of both antigen-specific and antiidiotypic T cell factors on the A cells could then favour the selection of clones that have the dual specificity of being antigen-specific and antiidiotypic. With respect to the antiidiotypic side of their specificity, we can expect the selected clones to be internal images of the antigen, since the more that the V regions resemble the antigen, the more they will be stimulated by various antigen-specific clones. This selection process for B cells to be both anti-BSA and BSA-mimicking specificity would account for the selection of very similar V regions in two such phylogenetically different species, namely chickens and mice. This model is not meant to mean that the anti-BSA part of a given

antibody V region is necessarily complementary to its own BSA mimicking part.

Hsu *et al.* studied the idiotypic connectivity of monoclonal antibodies specific for hen egg lysozyme (HEL). HEL is a small protein antigen, and in this respect is similar to BSA. Following a primary immunization (with complete Freund's adjuvant) or a secondary immunization (antigen in buffered saline),[147] monoclonal antibodies were isolated that had a high level of idiotypic connectivity to each other. None of the monoclonals reacted significantly with themselves, but there were many clones that reacted both with the antigen and with the idiotypes of some or even many of the other clones. Hence in this system there were again many antibodies that were both antigen-specific and antiidiotypic, where the idiotypes were those of other antigen-specific monoclonal antibodies.

Perturbing the repertoire by adding one antibody V region gene

A famous experiment by Weaver et al.[148] showed that a small perturbation of the immune system of a mouse at the level of a V region gene can have profound regulatory effects, of a form that would seem to be impossible to explain without invoking idiotypic network regulation. The experiment involved the NP system, consisting of the hapten NP[149], a well-characterized system of anti-NP antibodies with a particular idiotype, and anti-anti-NP (antiidiotypic) antibodies. An anti-NP hybridoma[150] expressing the idiotype was obtained and the heavy chain gene responsible for making the anti-NP antibodies was purified. This gene was then used to make transgenic mice[151] that had the gene, and the antibodies made by those mice were studied. It was found that the mice made high levels of antibodies with the idiotype associated

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147 D.-W. Hsu, E. E. Sercarz and A. Miller (1989) Internal connectivity is pervasive among primary and secondary anti-hen egg white lysozyme (HEL) IgG monoclonal antibodies. Int. Immunol. 1:197-204.

148 D. Weaver, H. Moema, M. H. Reis, C. Albanese, F. Costantini, D. Baltimore and T. Imanishi-Kari (1985) Altered repertoire of endogeneous immunoglobulin gene expression in transgenic mice containing a rearranged mu heavy chain gene. Cell, 45, 247-259.

149 (4-hydroxy-3-nitrophenyl) acetyl.

150 A hybridoma is a fused cell obtained from the fusion of a cancer cell and a lymphocyte, from which we can obtain unlimited amounts of antibody of a single specificity - in this case anti-NP with the particular idiotype.

151 A transgenic mouse is a mouse that has had a foreign gene incorporated into its genome.

with the transgene (the same idiotype as the original anti-NP antibodies, as defined by an antiidiotypic reagent), but the antibodies used endogenous heavy chains rather than the transgene. The presence of the transgene (with an idiotype that we will call Id-1) thus caused the production of antibodies with idiotypes that mimicked the transgene idiotype (say Id-1'). In order to account for this, it seems we would need to assume that the presence of Id-1 stimulates Id-2, which in turn selects the production of Id-1', where Id-1' is similar to Id-1. This is then an example of symmetry in stimulatory interactions between idiotypes and antiidiotypes *in vivo*. No one has been able to suggest a plausible alternative mechanism for this finding; it is a paradox in the context of clonal selection without an important role for idiotypic network interactions in the development of the repertoire.

The syngeneic barrier

When lymphocytes from an animal that is immune to an antigen X are injected into a non-irradiated recipient of the same strain, and the recipient is then immunized with X, the recipient fails to respond to the antigen.[152] This failure is mediated by T cells; it is not seen in recipient animals that lack T cells.[153] This can be explained as being due to co-selection of antigen-specific T cells in the transferred cells and antiidiotypic T cells in the recipient, leading to the suppressed state for the antigen. The syngeneic barrier is part of the reason for using irradiated recipients in adoptive transfer experiments. The other reason is that one typically wants to use the recipient as a "living test tube", that is initially empty, and one studies the interactions between the defined populations of cells that have been added.

Regulation of cytotoxic T cells

We have focused mainly on B cell immune responses and the ways in which they are regulated by helper T cells, suppressor T cells and contrasuppressor T cells. The regulation of cytotoxic T cell responses seems to have much in common with the regulation of B cells, with roles for helper and suppressor T cells. There is furthermore evidence supporting a specific helper T cell factor with serological determinants that map to the MHC class II region and a

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[152] F. Celada (1966) "Quantitative studies of the adoptive immunological memory in mice. I. An age-dependent barrier to syngeneic transplantation." J. Exp. Med. 124, 1-14.

[153] U. Kobow and E. Weiler (1975) Permissiveness of athymic ("nude") mice towards congenic memory cells. Eur. J. Immunol., 5, 628-637.

molecular weight in the range of 50,000 to 65,000[154,155]. A non-specific accessory cell is also involved[156], together with a non-specific factor.[157,158] With all these similarities to the regulation of B cells, it is reasonable to hypothesize that the model we have formulated for the regulation of B cells applies also to the regulation of cytotoxic T cells. There is more to be said about this however. As we will see in more detail in chapter 12, helper T cells and cytotoxic T cells have different repertoires. Helper T cells preferentially recognize one class of MHC determinants, while cytotoxic T cells have a preference for a different class of MHC.

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154 J. M. D. Plate (1976) Soluble factors substitute for T-T-cell collaboration in generation of T-killer lymphocytes. Nature 260, 329-331.

155 D. G. Kilburn, F. O. Talbot, H.-S. Teh and J. G. Levy (1979) A specific helper factor which enhances the cytotoxic response to a syngeneic tumour. Nature 277, 474-476.

156 R. G. Miller, H.-S. Teh, E. Harley and R. A. Phillips (1977) Quantitative studies of the activation of cytotoxic lymphocyte precursor cells. Immunol. Rev. 35, 38-58.

157 J. Shaw, V. Monticone, G. Mills and V. Paetkau (1978) Effects of costimulator on immune responses in vitro. J. Immunol. 120, 1974-1780.

158 P. L. Simon, J. J. Farrer and P. D. Kind (1977) The xenogeneic effect. III. Induction of cell-mediated cytotoxicity by alloantigen-stimulated thymocytes in the presence of xenogeneic reconstitution factor. J. Immunol.
118, 1129-1131.